Volume 18 Number 3

COMMONWEALTH OF AUSTRALIA

JOURNAL

OF

THE COUNCIL FOR SCIENTIFIC

AND

INDUSTRIAL RESEARCH

AUGUST, 1945

Registered at the General Post Office, Melbourne, for transmission by post as a periodical

C.6156/45.

Council for Scientific and Industrial Research

314 Albert Street,
East Melbourne,
Victoria

Volume 18 Number 3

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Registered at the General Post Office, Melbourne,
for transmission by post as a periodical

H. E. Daw, Government Printer, Melbourne

C.6156/45.

(PUBLISHED QUARTERLY)

Journal of the Council for Scientific and Industrial Research.

Vol. 18. AUGUST, 1945. No. 3.

CONTENTS.

Scientific Section.

General Section.

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The Influence of Dissolved Tin on the Growth of *Clostridium botulinum* in Canned Vegetables.

II. Further Experiments in Plain and in Lacquered Cans.

By W. J. Scott, B.Agr.Sc., and D. F. Stewart, B.V.Sc., Dip. Bact.†*

Summary.

Types A and B of both American and Australian strains of *Cl. botulinum* were inoculated into plain and lacquered cans of nine additional vegetables. Growth occurred consistently in lacquered cans of all vegetables. Inhibition of growth at 20°, 30°, and 37°C. for periods up to six months was observed in plain cans of french beans (cut green beans), silver beet (swiss chard), and parsnips in addition to beetroot and carrots previously reported. No inhibition was observed in plain cans of asparagus, cabbage, cauliflower, peas, potatoes, and white turnips. Inhibition of growth in plain cans was bacteriostatic, and was associated with concentrations of dissolved tin which exceeded the values for lacquered cans.

The concentrations of tin in solution which are necessary to prevent growth differ considerably between vegetables, and are positively correlated with reported protein contents. In some vegetables growth of *Cl. botulinum* in plain cans is probably determined by variations in the rates of dissolution of tin.

1. Introduction.

It has been reported previously (Scott and Stewart, 1944) that *Cl. botulinum* failed to grow when inoculated into internally plain cans of beetroot and carrots, and that inhibition of growth was explained by the bacteriostatic effect of dissolved tin. Investigations have been extended to other vegetables commonly canned in Australia and the results are reported in this paper.

2. Methods.

All inoculations were made in No. $2\frac{1}{2}$ cans of the various vegetables packed and processed according to the schedules recommended by the National Canners Association (1942), except potatoes which were processed according to the method of Hirst and Adam (1945). The methods were the same as those previously described with the following two exceptions. Firstly, inocula were standardized to 10,000 viable spores harvested from cultures in Robertson's cooked meat medium or neopeptone glucose broth, and secondly tin was determined by the method of McKenzie (1945). Vegetable liquors were not autoclaved prior to the analyses which were made on supernatant liquors after centrifuging in pyrex tubes. Unless otherwise specified inoculated cans were incubated at 30°C.

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Five strains of *Cl. botulinum* have been used. Australian strains L4 (type A) and 968 (type B) were isolated recently in these laboratories, and American National Canners Association strains 62_A (type A) and 213_B (type B) were obtained from Prof. K. F. Meyer. In addition a strain "type A" originally procured from America and maintained in Australia for some years was used in some experiments.

Cans were judged to be spoiled when microscopic examination of the liquor revealed an abundance of organisms, in contrast to unspoiled cans which showed the virtual absence of bacteria. The results of microscopic examinations were confirmed by culturing and by toxin-antitoxin tests with the liquors injected into mice. The presence of viable spores was demonstrated in unspoiled cans by deep cultures of liquor heated at 80°C. for 10 minutes.

3. Results.

(i) *Inoculation of Plain and Internally Lacquered Cans.*

The study of the growth of *Cl. botulinum* in plain and in internally lacquered cans was extended to the following vegetables: asparagus, cabbage, cauliflower, french beans (cut green beans), parsnips, peas (green), potatoes, silver beet (swiss chard), and white turnips.

Typical results, shown in Table 1, indicate that growth occurred in all lacquered cans. In plain cans, however, *Cl. botulinum* failed to grow in french beans, parsnips, and silver beet during incubation for three weeks. The findings in these three vegetables were similar to those in beetroot and carrots (Scott and Stewart, 1944), and the absence of growth in plain cans was associated with greater amounts of dissolved tin than in the lacquered cans.

An examination of Table 1 shows that vegetables vary widely in their capacity to dissolve tin from plain cans. Although asparagus and potatoes dissolve the greatest amounts of tin amongst these vegetables the growth of *Cl. botulinum* is not inhibited. The inhibition of growth by dissolved tin is discussed subsequently in relation to the composition of the vegetable.

Cl. botulinum produces a characteristic off-odour manifest to a degree varying with the vegetable in which it has grown. In vegetables of high protein content the odour is marked, but in products such as parsnips, silver beet, and white turnips inexperienced observers may have difficulty in detecting any abnormality. It will be noted that in parsnips and white turnips some of the spoiled cans remained flat.

(ii) *Incubation of Inoculated Plain Cans at 37°, 30°, and 20°C.*

As inhibition of growth in plain cans might well be influenced by the rate of spore germination and the rate of accumulation of dissolved metals, it was considered desirable to incubate inoculated plain cans for relatively long periods at various temperatures favourable for the growth of *Cl. botulinum.* These experiments were carried out in beetroot, carrots, french beans, parsnips, and silver beet in which inhibition in plain cans had been observed. Plain cans of each vegetable were inoculated with 10,000 spores of type A strains L4 and 62_A and type B strains 968 and 213_B, one can of each being incubated at 20°, 30°, and 37°C. Inoculations were made within 24 hours of processing. The results are summarized in Table 2.

All cans of beetroot and carrots remained flat throughout the incubation period of six months and none was spoiled. This confirmed the previous results in these vegetables with shorter periods of incubation at 37°C. (Scott and Stewart, 1944). In beetroot the analyses of composite aliquots from the liquors of the four cans incubated at 37°C. was 168 p.p.m. of tin, at 30°C. 194 p.p.m., and at 20°C. 204 p.p.m.

TABLE 1.—INOCULATION OF PLAIN AND LACQUERED CANS.

Vegetable.	Internal Surface of Can.	Strain Inoculated.	Incubation at 30° C.	Spoilage.	Tin Content of Liquor.	Initial pH.	Final pH.
			days		p.p.m.		
Asparagus (cuts)	Lacquered	L4 ..	4	Yes S*	14	5·43	5·43
		968 ..	4	Yes S	11	5·36	5·43
	Plain ..	L4 ..	5	Yes S	106	5·33	5·43
		968 ..	4	Yes S	138	5·29	5·42
Cabbage ..	Lacquered	Type A	6	Yes S	9	5·24	5·00
		968 ..	6	Yes S	12	5·21	4·93
	Plain ..	Type A	6	Yes S	19	5·24	5·28
		968 ..	14	Yes S	24	5·17	5·01
Cauliflower	Lacquered	L4 ..	4	Yes S	9	5·46	5·14
		968 ..	4	Yes S	14	5·43	5·24
	Plain ..	L4 ..	5	Yes S	10	5·40	5·11
		968 ..	8	Yes S	42	5·33	5·31
French Beans (cut)	Lacquered	L4 ..	8	Yes S	11	5·21	5·21
		968 ..	7	Yes S	10	5·21	5·36
	Plain ..	L4 ..	20	No F†	26	5·21	5·16
		968 ..	20	No F	27	5·20	5·16
Parsnips (diced)	Lacquered	L4 ..	8	Yes S	10	5·12	4·96
		968 ..	14	Yes F	13	5·09	5·00
	Plain ..	L4 ..	21	No F	26	5·07	5·00
		968 ..	21	No F	21	5·14	5·00
Peas ..	Lacquered	Type A	2	Yes S	33	5·85	6·00
		968 ..	2	Yes S	16	5·82	6·45
	Plain ..	Type A	2	Yes S	27	5·95	6·63
		968 ..	2	Yes S	25	6·05	6·52
Potatoes (diced)	Lacquered	L4 ..	5	Yes S	20	5·55	5·49
		968 ..	5	Yes S	21	5·48	5·39
	Plain ..	L4 ..	5	Yes S	49	5·53	5·21
		968 ..	5	Yes S	97	5·58	5·13
Silver Beet	Lacquered	L4 ..	14	Yes S	16	5·13	4·97
		968 ..	9	Yes S	34	5·19	5·14
	Plain ..	L4 ..	21	No F	61	5·16	5·21
		968 ..	21	No F	82	5·08	5·21
White Turnips (diced)	Lacquered	L4 ..	53	Yes F	6	4·91	4·80
		968 ..	15	Yes S	2	4·96	5·05
	Plain ..	L4 ..	53	Yes F	13	4·99	4·80
		968 ..	53	Yes F	16	4·91	4·89

*S = Swelled Can. †F = Flat Can.

TABLE 2.—INCUBATION OF INOCULATED PLAIN CANS AT 37°, 30°, AND 20°C.

Product.	Incubation.		Number of Cans.		Final pH.	Sn Content Centrifuged Liquor	Remarks.
			Spoiled.	Un-spoiled.			
	Deg. C.	Days.				p.p.m.	
Beetroot (diced)	37	180	..	4	4·77–4·86	168	
	30	180	..	4	4·90–5·02	194	
	20	180	..	4	5·15–5·28	204	
Carrots (diced)	37	182	..	4	4·72–4·88	41	
	30	182	..	4	5·00–5·00	32	
	20	182	..	4	5·10–5·14	28	
French Beans (cut)	37	17	1	..	5·31	24	Swelled in 17 days. Strain 213B
		184	1	..	5·02	72	Springer after 19 days. Strain 968.
		184	..	2	4·98–5·07	80	Strains L4 and 62A
	30	184	..	4	5·07–5·09	54	
	20	184	..	4	5·23–5·28	38	
Parsnips (diced)	37	86	..	4	4·99–5·00	35	
	30	86	..	4	5·00–5·07	28	
	20	86	..	4	5·10–5·19	19	
Silver Beet, Series 1	37	5	1	..	5·54	6	Swelled in 5 days. Strain L4.
		5	1	..	5·47	7	Swelled in 5 days. Strain 62A
		17	1	..	5·21	10	Swelled in 17 days. Strain 968.
		185	..	1	5·10	69	Strain 213B
	30	7	1	..	5·86	9	Swelled in 7 days Strain L4.
		11	1	..	5·35	6	Swelled in 11 days. Strain 968.
		12	1	..	5·23	11	Swelled in 12 days. Strain 62A
		31	1	..	5·00	34	Swelled in 31 days. Strain 213B
	20	20	1	..	5·42	10	Swelled in 20 days. Strain 213B
		185	1	..	5·15	70	Flat Can. Strain 62A
		185	..	2	5·25–5·28	43	Strains 968 and L4.
Silver Beet, Series 2	37	172	..	4	5·14–5·17	155	
	30	172	..	4	5·10–5·30	148	
	20	172	..	4	5·30–5·47	149	

In carrots the corresponding figures were 41 p.p.m., 32 p.p.m., and 28 p.p.m.

Of the twelve cans of french beans, ten were unspoiled and two, incubated at 37°C., spoiled. Of the two cans showing growth of *Cl. botulinum* one can swelled after seventeen days. It was opened immediately, and analysis of the liquor revealed a tin content of 24 p.p.m. The second can became a "springer" after nineteen days, but was incubated for the full period of six months. There was no further advance in the swelling of the can which remained a "springer." When opened the tin content of the liquor was found to be 72 p.p.m., a figure considerably in excess of the first can. There is no doubt that tin continued to accumulate after the time (nineteen days) when evidence of growth first was indicated by the "springer" condition of the can, and it is possible that the increased concentration of tin was responsible for the apparent cessation of gas production. In the remaining two cans at 37°C. the level of tin in composite aliquots of the liquors was 80 p.p.m. None of the cans incubated at 30° or 20°C. was spoiled, and tin levels in composite samples were 54 p.p.m. and 38 p.p.m. respectively.

Cl. botulinum failed to grow in all twelve cans of parsnips after incubation for approximately three months. The concentration of tin in composite aliquots was 35 p.p.m. at 37°C., 28 p.p.m. at 30°C., and 19 p.p.m. at 20°C. It must be stated, however, that there is some variation in parsnips and growth in plain cans has been observed when dissolution of tin has been slow, not exceeding 12 p.p.m. after four months.

Two series of silver beet were inoculated and the results obtained present a marked contrast. In the first series most of the cans swelled within a month and the levels of dissolved tin were low. Only four cans remained flat, and of these one, incubated at 20°C., was spoiled. None of the cans in the second series spoiled during six months incubation. The liquors of the cans at 37°C. contained 155 p.p.m. of tin, at 30°C. 148 p.p.m., and at 20°C. 149 p.p.m. These figures are more than twice as great as those for the cans of the first series incubated for a comparable period.

It will be noted that there were no consistent differences in the behaviour of the four strains inoculated.

(iii) *The Effect of Added Tin on Growth in Lacquered Cans.*

In these experiments graded amounts of the stannous chloride-sodium citrate solution were added at the time of inoculation. To each of two series of five or six lacquered cans of each vegetable, tin solution was added in steps of 30 or 60 p.p.m., one series being inoculated with strain L4 and the other with strain 968. The quantities of tin solution added were equivalent to the stated concentration in p.p.m. assuming uniform distribution throughout a volume of 800 ml. Recoveries of added tin were accurate to 5 per cent. (McKenzie, 1945). In each series was included a control can without added tin to which was added sodium citrate equivalent to that added in the greatest amount of the stannous chloride-sodium citrate solution. The cans were incubated at 30°C. and "swells" were removed for examination as they developed. Flat cans were incubated for twelve weeks.

Close agreement was obtained with the two strains inoculated into each vegetable, and Table 3 sets out the results. The addition of tin in concentrations up to 300 p.p.m. failed to inhibit growth in either peas or asparagus. The concentrations of added tin which prevented growth were 300 p.p.m. in cauliflower, 240 p.p.m. in potatoes, 180 p.p.m. in cabbage, 90 p.p.m. in french beans, and 60 p.p.m. in white turnips. In parsnips some variation was encountered and in one series growth occurred with 120 p.p.m. of added tin, whereas in another series growth was prevented by the addition of 60 p.p.m.

It will be noted that in each vegetable the added tin has not all remained in solution. For instance in potatoes more tin remained in solution when 180 p.p.m. was added than in cauliflower when 300 p.p.m. was added. Again there is a difference between the two series of parsnips. In silver beet the amount of tin remaining in solution after three months was generally less than 20 per cent. of the amount added, and it has not been possible to obtain inhibition regularly over this period by adding the tin-citrate solution to this vegetable.

TABLE 3.—INOCULATION OF LACQUERED CANS WITH ADDED TIN INCUBATED AT 30°C. FOR TWELVE WEEKS.

Vegetable.	Concentration of Added Tin p.p.m.							
	0.	30.	60.	90.	120.	180.	240.	300.
Peas	+	..	..	..	+	+	+	+
Asparagus (cut)	+	..	+	..	+	+	+	+
Cauliflower	+ (2)*	..	+ (32)	..	+ (71)	+ (91)	+ (94)	— (105)
Potatoes (diced)	+ (4)	..	+ (40)	..	+ (83)	+ (132)	— (162)	— (200)
Cabbage	+ (2)	+ (21)	+ (39)	..	+ (61)	— (83)	— (100)	..
Parsnips (diced), Series 1	+ (1)	+ (14)	+ (28)	+ (40)	+ (51)	..	..	..
Parsnips (diced), Series 2	+ (2)	..	— (40)	..	— (70)	— (98)	— (131)	— (157)
French Beans (cut)	+ (2)	..	+ (14)	— (28)	— (40)	— (50)	..	..
White Turnips (diced)	+ (2)	+ (25)	— (48)	— (69)	..	..	..	..

+ = Cans spoiled by *Cl. botulinum* strains L4 and 968.
— = Cans not spoiled by *Cl. botulinum* strains L4 and 968.
.. = Not done.
* = Figures in parenthesis refer to mean tin contents (p.p.m.) of centrifuged liquors.

4. Discussion.

Inhibition of growth of *Cl. botulinum* inoculated into internally plain cans of beetroot and carrots has been confirmed during extended periods of incubation at 20°, 30° and 37°C. Investigation of nine other vegetables has shown that *Cl. botulinum* may also fail to grow in plain cans of french beans, parsnips, and silver beet. In all products growth has been consistent in internally lacquered cans, and failure to grow in plain cans has been associated with greater quantities of dissolved tin. The inhibitory effect of tin has also been observed when a tin-citrate solution was added to lacquered cans.

In each vegetable, however, the concentration of dissolved tin required for inhibition differs considerably. While there is as yet no explanation for these differences it may be significant that the concentration of tin required increases with the protein content of the vegetable. For example, growth was not inhibited by the addition of 300 p.p.m. of tin to peas and asparagus in which the protein contents have been reported to be 5·8 and 3·4 per cent. respectively (McCance and Widdowson, 1942). On the other hand growth was prevented in french beans by the addition of 90 p.p.m. of tin and in carrots and white turnips by 60 p.p.m., containing 0·8, 0·7, and 0·7 per cent. protein respectively. The vegetables of intermediate protein contents required intermediate quantities of tin to produce inhibition of *Cl. botulinum*. In the vegetables in which inhibition was achieved, a significant correlation ($r = + 0{\cdot}82$, $p = 0{\cdot}02$) exists between the protein contents and the levels of dissolved tin required to prevent growth. This relationship between tin and protein contents would be consistent with the reversal of bacteriostatic action by sulphydryl compounds, as shown by Fildes (1940) for the bacteriostatic effect of mercury.

Another factor which will influence the chances of growth in plain cans is the rate of dissolution of tin. Various products are known to vary widely in their ability to dissolve tin, and this capacity is probably dependent on the concentrations of significant anions such as citrate, oxalate, tartrate, and others which are able to combine to form soluble tin complexes. Even in any one product, however, there may be substantial variations in the rate of dissolution and many factors, including the type and condition of the tinplate, pH, and the degree of vacuum, are known to be important. For these reasons considerable variations in the rates of dissolution of tin are to be expected in different commercial lots of any one vegetable. The chances of growth in plain cans will depend upon the relative rates of spore germination and accumulation of tin to the level inhibitory in the particular product, and the factors controlling the rate of dissolution of tin might well determine the outcome.

In plain cans of french beans, parsnips, and silver beet inhibition has not been as regular as in carrots and beetroot. In french beans the amount of tin dissolved was not greatly in excess of the amount required for inhibition, and *Cl. botulinum* was able to develop in occasional cans. Different batches of silver beet varied substantially in their tin concentrations (Table 2), and a similar though not so marked variation occurred with parsnips. In these vegetables growth of *Cl. botulinum* has occurred only when the accumulation of tin was slow, and variation

in the rate of dissolution has probably governed the development of the inoculum. The amounts of tin dissolved from plain cans of peas, asparagus, potatoes, cauliflower, cabbage, and white turnips have been insufficient to prevent growth of *Cl. botulinum*. It should be noted, however, that in white turnips the margin between the amount dissolved and the added tin required for inhibition was small.

In all cans in which *Cl. botulinum* failed to grow, viable spores were demonstrated in cultures, and there can be little doubt that the effect of dissolved tin is bacteriostatic. However, the mechanism by which tin is inhibitory, the chemical form in which dissolved tin occurs naturally in various products, and the factors governing its dissolution are matters for further study before the conditions determining the growth of *Cl. botulinum* in plain cans can be defined. Nevertheless there is need for considerable care in the interpretation of incubation trials with foods packed in plain cans in which the product may consistently, or even occasionally, dissolve sufficient tin to interfere with the growth of *Cl. botulinum.*

5. Acknowledgments.

Canned vegetables were prepared by the Canning Section of this Division and tin analyses were made by the Chemistry Group.

6. References.

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A Volumetric Method for the Determination of Tin in Foods.

*By Hugh A. McKenzie, B.Sc.**

Summary.

Methods for the determination of tin in foods are discussed, and a method involving wet digestion and iodometric titration is described.

The food is digested with nitric and sulphuric acids and hydrogen peroxide. An aliquot of the digest (preferably containing 0·5 to 5 mg. of tin) is reduced by aluminium and titrated with N/200 potassium iodate. A dilute potassium iodide soluton containing sodium bicarbonate is added immediately prior to titration.

Recovery tests in pure solution in the absence and presence of iron and copper show the precision and reproducibility of the method to be of the order of ± 3 per cent. Tests have also been carried out for orange juice and vegetables.

1. Introduction.

Recent work in this laboratory (Scott and Stewart, 1944, 1945) on the influence of dissolved tin on the growth of *Clostridium botulinum* required large numbers of accurate determinations of small amounts of tin in canned foods. Many of the methods used in the past were designed to determine the tin content of foods in relation to legal limits (285 p.p.m. in Great Britain and Australia and 300 p.p.m. in the United States of America), and great accuracy at low tin contents was not sought. The method of Lynch and Kefford (1939) originally used in this laboratory was of this type.

Park (1934) and Staud (1936) have estimated small amounts of tin by the spectrographic method. This method is particularly advantageous when other metals are to be determined simultaneously, but the necessary equipment is not often available. The difficulties associated with the polarographic reduction of tin have been discussed by Lingane (1943). Colorimetric procedures have been developed by Tartakovskiĭ (1940), Clark (1936, 1937), Schwaibold, Borchers, and Nagel (1940), De Giacomi (1940), Stone (1941), Law (1942), and Baker, Miller, and Gibbs (1944). Adam and Horner (1937) employed a gravimetric procedure. However, gravimetric and colorimetric procedures involve separation of the tin by difficult and tedious precipitation methods or by distillation.

Therefore it was considered that a volumetric method would be most suitable for the purpose in view. A procedure involving wet digestion, reduction with aluminium, and titration with potassium iodate in the presence of iodide, is discussed.

2. Basis of the Method.

(i) *Destruction of Organic Matter.*

Wet digestion with nitric and sulphuric acids is preferred for products containing tin since dry ashing results in difficultly soluble oxides and the high temperature employed may permit appreciable loss of tin. Economy in the use of reagents is secured by adding a small volume of nitric acid initially and additional small volumes subsequently

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(cf. Lench and Pilkington, 1943). The digestion is completed with hydrogen peroxide to remove traces of nitric acid or its reduction products.

(ii) *Reduction.*

Several metals such as nickel, antimony, iron, zinc, lead and aluminium have been used as reductants, and their merits and defects discussed (Powell, 1918; Oesterheld and Honegger, 1919; Jilek, 1923; Sandved, 1927; and Clarke, 1931). Evans (1931) proposed the use of hypophosphorous acid as reductant. Aluminium has been used in this laboratory as it dissolves completely during the reduction and does not interfere with the subsequent titration.

The glass apparatus devised for the reduction incorporates a sodium bicarbonate suck-back which performs two functions. The bicarbonate drawn back reacts with the acid in the flask, generating carbon dioxide to maintain an inert atmosphere and at the same time the acidity is lowered by this reaction and by dilution. McDow *et al.* (1944) applied dry ice in this connexion to cool the liquid and to generate carbon dioxide.

(iii) *Titration.*

In the iodometric determination of tin the following procedures are applicable:—

(i) Direct titration with iodine-iodide, with iodate-iodide or with iodate alone; or

(ii) back titration with thiosulphate after addition of excess of any one of these reagents.

In the present work, addition of excess N/200 iodine followed by back titration with N/200 thiosulphate gave many anomalous results even in the determination of reagent blanks. Errors of ± 20 per cent. were common, and blanks varied from 0·1 to 0·5 ml. of N/200 iodine (about 1 to 7 parts of tin per million of sample analysed). These observations indicated that the iodine reacted slowly with other substances beside stannous tin, and for this reason a direct titration was tried. The iodometric oxidation of stannous salts in acid solution appears to be practically instantaneous. When an excess of iodine is present for an appreciable period sluggish iodine-consuming reactions have time to take place.

Okell and Lumsden (1935), in direct titrations of tin with N/100 iodine in the presence of M/5 potassium iodide, obtained low results which they attributed to oxygen dissolved in the reagents. Accordingly they adopted titration with air-free iodine solutions and claim that the stannous-iodine reaction is then stoichiometric. This procedure involves elaborate preparation of reagents, and is inconvenient for regular use.

Since iodide and hydrogen ions catalyse the stannous-oxygen reaction, Ramsey and Blann (1934) used potassium iodate alone as a titrant (N/10 iodate for the titration of about 25 ml. of N/10 tin), and obtained satisfactory results. The acid concentration was low (less than 3N HCl) and dissolved oxygen was not removed from the iodate solution. Iodate has important advantages in being a primary standard and stable in solution. In the present work, direct titrations of small amounts of tin with N/200 potassium iodate gave results which were

much too high. Errors of + 50 per cent. in the titration of 0·6 mg. of tin (30 p.p.m.) were quite common. However, it was then found that satisfactory results were obtained if a little dilute potassium iodide solution was added immediately prior to titration.

3. Recommended Procedure.

The procedure finally adopted for determination of tin in foods is given below.

(i) *Reagents.*

High grade reagents must be employed throughout and blank determinations carried out. With the reagents employed in the investigations, the blank was never greater than 0·15 ml. of N/200 iodate.

Hydrochloric acid—3N.

Sulphuric acid—36N.

Nitric acid—16N.

Hydrogen peroxide—30 per cent. (100 volume).

Aluminium foil—Purest possible grade.

Sodium bicarbonate—5 g. in 100 ml. of water.

Potassium iodide—Dissolve 0·2 g. potassium iodide and 3 g. sodium bicarbonate in 100 ml. of boiled-out water and transfer to a small reagent bottle. Add a few drops of dilute hydrochloric acid and agitate the contents. When the reaction has ceased insert the stopper and remove aliquots when required.

Potassium iodate—Make an N/200 solution by dilution of N/10 stock solution with boiled-out water. Store in the volumetric flask in which it is prepared, and connect to a 10 ml. (semi-micro) burette by siphon.

The potassium iodide and iodate reagents should be prepared at least once a day.

Starch indicator—1 per cent. soluble starch in 20 per cent. sodium chloride.

(ii) *Apparatus.*

The only special apparatus required is the reduction vessel which consists of a 150 ml. B24 Quickfit conical flask, into which is fitted a B24 ground glass cone connected to a length of pyrex capillary tube (2mm. internal bore, 6 mm. external). This tube is bent at right angles about 9 cm. from the upper end of the neck of the flask and again at right angles about 10 cm. from the first bend. The end of the capillary dips into a vessel containing sodium bicarbonate solution. The size of capillary suggested is found to give good response to changes in pressure. Six reduction flasks are set up on a suitable stand with a bunsen burner under each. The apparatus is illustrated in Fig. 1.

(iii) *Procedure.*

(*a*) *Sampling.*—50 g. samples are usually taken. Two procedures were applied to obtain representative samples from solid-liquid canned foods. The product was homogenized in a Waring Blendor before sampling, or alternatively the solids were drained on a stainless steel screen and a composite sample of solids and liquids taken according to their weights. Liquid foods present no sampling difficulty.

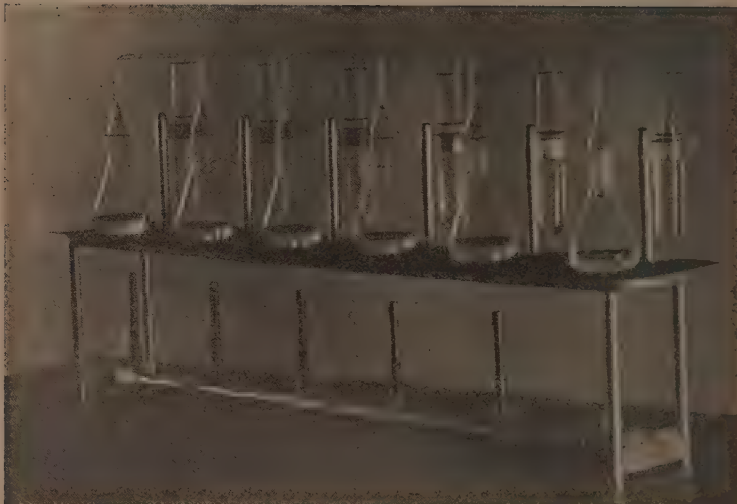

FIG. 1.—Apparatus for Reduction of Tin Solutions.

(*b*) *Digestion.*—Mix the sample of food (usually 50 g.) with nitric acid (20 ml.) and sulphuric acid (10 ml.) in a Kjeldahl flask (500 ml.) and add a glass bead to prevent bumping during digestion. Heat gently at first to prevent undue loss of nitric acid and then more strongly until charring commences and white fumes appear. Allow the mixture to cool until fuming just ceases, and add nitric acid (5 ml.) dropwise. Heat again until white fumes appear. Further additions of nitric acid may be necessary if the material is difficult to digest. Allow the flask to cool as before, and add hydrogen peroxide (10 ml.) dropwise. Heat again till white fumes appear. Add further peroxide if necessary, until colourless. For most foods the process of digestion takes about two hours, and about one hour for liquors from canned vegetables. If a white precipitate of calcium sulphate appears on cooling the digest, redissolve by adding water and reheating. Make up the digest to 50 ml. with water. The diluted digest may appear slightly turbid but it is sufficiently homogeneous for measuring aliquots, and the turbidity disappears during subsequent reduction.

(*c*) *The reduction and titration.*—Transfer an aliquot (20 ml.) of the digest to a reduction flask. Add hydrochloric acid (30 ml.) and aluminium foil (12 pieces, each approximately 1 x 1 cm. and 0·002 inch thick, total weight 0·3 g.). Fit the flask with the delivery tube dipping into the bicarbonate solution, heat gently until evolution of hydrogen has started and then discontinue. When the aluminium has nearly all dissolved apply heat again and, finally, boil until the liquid is perfectly clear. Remove by gentle agitation any metal particles remaining at the liquid-glass interface. Cool the flask in ice water to below room temperature. When cool wash down the walls with approximately 4 ml. of iodide-carbonate reagent run in quickly from a 5 ml. pipette with the tip removed. Add a few drops of starch indicator and titrate the tin with N/200 iodate solution until the iodine blue first appears.

4. Recovery Tests.

The accuracy and reproducibility of the suggested procedure were examined by recovery tests on pure tin solutions and on foods containing known amounts of tin. Recovery tests were also carried out in the presence of added iron and copper to examine the possibility of interference by these metals which commonly occur in foods.

Standard tin solutions in 3N hydrochloric acid were prepared from Analar tin and Analar stannous chloride. A standard tin citrate solution was prepared by dissolving stannous chloride in sodium citrate solution in the ratio one mole of stannous chloride to two moles of sodium citrate.

Using these standard tin solutions, the following recovery tests were made:—

1. An aliquot (20 ml.) of the standard tin chloride solution was added to the reduction flask followed by hydrochloric acid (10 ml.), sulphuric acid (20 ml. 6N) and aluminium foil, and the tin content determined by reduction and titration. The results are given in Table 1.

TABLE 1.—RECOVERY TESTS WITH PURE TIN SOLUTIONS.

Tin Added.	No. of Detns.	Tin Found.*	
		Mean.	S.D.
Mg. in 20 ml.		Mg. in 20 ml.	Mg. in 20 ml.
0·6	6	0·60	0·02
1·2	5	1·19	0·02
2·4	4	2·39	0·04
4·8	6	4·78	0·04

2. Aliquots of standard tin solution were digested with nitric and sulphuric acids and hydrogen peroxide and the tin content determined. The results are given in Table 2.

TABLE 2.—RECOVERY TESTS WITH PURE TIN SOLUTIONS AFTER DIGESTION.

Tin Added.		Iron Added.	Copper Added.	No. of Detns.	Tin Found.	
					Mean.	S.D.
Mg. in 20 ml.	p.p.m. (50 g. sample)	Mg. in 20 ml.	Mg. in 20 ml.		Mg. in 20 ml.	Mg. in 20 ml.
0·6	30	..	..	6	0·60	0·02
2·4	120	..	..	6	2·39	0·01
4·8	240	..	..	6	4·74	0·08
0·6	30	4	..	4	0·60	0·02
0·6	30	4	0·4	4	0·60	0·02
2·4	120	4	0·4	4	2·42	0·03

* *Note*:—Blanks have been subtracted. The standard deviation is taken as $\sqrt{\frac{\Sigma d^2}{n-1}}$ and the standard deviations (S.D.) tabulated include the standard deviations of the blanks.

3. An aliquot of tin solution was added to 50 g. of fresh orange juice, which was then analysed for tin according to the recommended procedure. The results are given in Table 3.

TABLE 3.—RECOVERY TESTS FOR TIN ADDED TO ORANGE JUICE.

Tin Added.		Iron Added.	Copper Added.	Tin Found (Mean of 2 Detns.).
Mg. in 20 ml.	p.p.m. (50 g. sample)	Mg. in 20 ml.	Mg. in 20 ml.	Mg. in 20 ml.
0·6	30	..	..	0·59
2·4	120	..	..	2·35
0·6	30	4	0·4	0·61
2·4	120	4	0·4	2·40

4. In connexion with the bacteriological investigation mentioned above, recovery tests were made on known amounts of tin added to vegetables packed in lacquered No. 2½ cans. Processed cans were heated, then punctured, tin citrate solutions added aseptically and the cans immediately resealed with solder. After storage for periods of the order of three months the can contents were analysed for tin. The results are given in Table 4.

TABLE 4.—RECOVERY TESTS FOR TIN ADDED TO LACQUERED CANS CONTAINING VEGETABLES.

Vegetable.	Tin Content.			Recovery.
	Added.	Found (Mean of 2 Detns.).		
	Mg. per can.	Mg. per can.	p.p.m.	Per Cent.
Cabbage	180	171	224	95
Beetroot	68	71	86	105
Carrot	23	25	30	109
French Beans	68	73	94	108
Silver Beet	90	98	130	109
Parsnip	45	47	58	104
Potato	132	131	156	99
Cauliflower	222	216	268	97
Turnip	45	47	58	104

5. Discussion.

Examination of the results of the recovery tests set out in Tables 1, 2, and 3 show that the precision and reproducibility of the method is of the order of ± 3 per cent.

Determination of 0·6 mg. (30 p.p.m.) of tin in the presence of 4 mg. (200 p.p.m.) of iron, or 4 mg. of iron and 0·4 mg. (20 p.p.m.) of copper, gave results which were not significantly different from those when iron and copper were absent. Since no marked interference was observed at this level of tin it is not likely to occur at higher levels of tin.

In the presence of 2 mg. (100 p.p.m.) of copper, or 2 mg. of copper and 4 mg. (200 p.p.m.) of iron, results were sometimes high and sometimes low, to the extent of $\pm$ 10 to 20 per cent. At this high level of copper a considerable amount of copper was plated out during the reduction and would not redissolve. The deposited copper probably contains appreciable amounts of tin. This observation and inference have been confirmed recently by Evans and Higgs (1944A, B). In the present work accurate results have been proved in the presence of at least as much as 0·4 mg. (20 p.p.m.) of copper, a copper content which is rarely exceeded, even in canned tomato products.

Table 4 shows an average difference between the amounts of tin added and recovered of about 5 per cent. It is to be emphasized that in this case the tin was added to foods in cans and therefore the test is not as critical as the others.

The volumetric method described has determined tin contents in foodstuffs with satisfactory precision and reproducibility. No difficult separations are involved, and no elaborate precautions in preparing and storing reagents are required. Several hundred analyses on a wide variety of products have now been carried out according to this procedure by the author and other workers.

6. Acknowledgments.

Grateful acknowledgment is due to Dr. F. E. Huelin and Mr. J. F. Kefford for the interest they have taken in this work, and to Mr. R. A. Gallop for carrying out confirmatory analyses.

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The Frictional Properties of Wool Fibres.*

By E. H. Mercer, B.Sc.†

Summary.

A method of studying the frictional properties of single wool fibres, based on the stick-slip method of measuring friction devised by Bowden and Leben, is described.

Measurements on the friction of wool fibres show that the coefficients of friction vary with the pH wetting solutions. The surfaces of the fibres exhibit a frictional difference between rootward and tipward motion, which is the cause of the unidirectional migration of fibres during felting. The frictional difference increases in acid and alkaline media, and this is held to account in part for the more rapid rate of felting in such media.

Several shrinkage-reduction ("unshrinkable") processes are found to reduce the frictional difference. This is the probable cause of their reducing the rate of felting.

The physical nature of the friction developed between fibres and other surfaces is discussed.

1. Introduction.

The solution of several problems in connexion with the felting of wool requires an investigation of the frictional properties of the individual wool fibres. The situation can be best understood from a brief account of the theories which have been advanced to account for felting. The original theories associated with the names of Shorter (1923) and Arnold (1929) emphasized the role of the directional friction in promoting the unidirectional migration of fibres, which leads to the tangling of the fibres and to consolidation of the fabric. The term directional friction was introduced by Martin (Anon. 1941) to designate that type of friction in which the coefficient depends upon the direction of relative movement of the surfaces in contact; it is used throughout this paper in this sense. It is a property displayed by many surfaces of animal origin and its probable biological importance has been discussed by Martin (1944). The original term "scaliness," applied by Speakman and Stott (1931) to the directional friction of hairs and wool fibres, has the defect that it supposes a specific mechanism, viz., imbricated scales, for the unsymmetrical friction. Some of the phenomena associated with directional friction described by Martin, and others, which are to be described in this paper, seem too complex to be attributed to the mechanical effect of cuticular scales unless the properties attributed to the scales be greatly elaborated. The less specific term "directional friction" is therefore preferable until more experimental data have been obtained.

The relation between the rate of felting (measured by shrinkage of fabric during milling) and the pH of the felting bath was determined by Speakman, Stott, and Chang (1933). They observed that the rate of felting and the extensibility of fibres varied in a similar way with pH and concluded that the fibres stretched while migrating during the progress of felting. The decrease of felting rate at pH values higher than 10 and at temperatures greater than 45°C., was taken as proof that contraction after stretching also occurred. Martin (1944; Anon., 1941)

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* Typescript received April 17, 1945.

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considered that the directional friction of the fibres was the controlling variable rather than the elasticity of the fibres. Mercer (1942), using the shrinkage of yarn lengths as a criterion of felting, found discrepancies between the observed shrinkages and those to be expected from the theory that fibres stretched and contracted during migration. For instance, the same amount of felting was found when yarns were soaked for hours in the felting bath, as when they were wetted and washed at once. In the case of immediate washing, the elasticity of the fibres would be practically the same as that of air-dried wool, and therefore it could not be the variable chiefly determining the rate of felting under these conditions.

To some extent the difference in view can be reconciled by distinguishing between "light" felting and "heavy" felting. If we define "light felting" as a process effected by a succession of small forces (i.e., forces too small to extend the fibres by more than 1 or 2 per cent.) then we can expect directional friction to be the major variable. This type of felting probably takes place during the normal washing of woollen materials. In "heavy felting" forces large enough to stretch the fibres are assumed and the resultant felting rate would presumably be a function of the two variables: elasticity and directional friction. The felting produced by the milling procedure adopted by Speakman and his colleagues would be of this type.

To assist in the further clarification of the problem, data relating the directional friction to the pH of the felting bath, obtained independently of felting experiments, are obviously necessary.

The other outstanding problem related to the felting of wool is a detailed understanding of the *modus operandi* of the felting or shrinkage-reduction ("unshrinkable") processes which have been developed in recent years. The many chemical treatments which have been found to reduce the felting rate of wool may be divided into two classes: (1) those which propose to alter the extensibility of the fibres, and (2) those which ideally limit their attack to the fibre surface and presumably alter the frictional properties of the fibres. With this second class, we are concerned in this paper.

Since the completion of these experiments, a letter by Whewell, Rigelhaupt, and Selim (1944) has appeared describing some experiments on the reduction in directional friction produced by chlorine treatment. A brief account of the present work has also appeared (Mercer, 1945).

2. The Measurement of the Frictional Properties of Fibres.

The first attempts to measure the friction of wool fibres were made by Speakman and Stott (1931) and Speakman, Stott, and Chang (1933). These authors made use of the well-known "angle of slip" method. Fifty fibres were stretched across a surface and the angle of slip of this preparation on an inclined plane covered by a piece of wool velvet was determined. The method was not very easy to handle, but it was demonstrated that the different felting properties of wools could be correlated with their frictional properties. Speakman *et al.* defined a "scaliness coefficient" as $\frac{\mu_1 - \mu_2}{\mu_1} \times 100$ where μ_2 and μ_1 are the coefficients of friction against and with the scales respectively. The rates of felting for many wools were found to be closely correlated with the scaliness coefficient.

This method has been used again recently by Whewell, Rigelhaupt, and Selim (1944) in their investigation on the changes in scaliness produced by chlorination.

Martin (Anon., 1941) described a qualitative method of detecting the presence of directional friction. A short piece of fibre was placed between two pieces of glass which were then caused to oscillate relative to each other. Directional friction was shown by the migration of the fibre. Using this simple apparatus, Martin made several important observations which led him to conclude that the directional friction was of greater importance than previously considered. Chamberlain and Speakman (1942) described an apparatus which is in principle a quantitative development of Martin's method. The actual migratory force exhibited by a fibre held between two oscillating surfaces is measured. The method is capable of yielding useful information about directional friction, but from the more general viewpoint of the theory of friction, it has two defects: the actual load applied between the rubbing surfaces is uncertain, and a separate determination of μ_2 and μ_1 is not made.

The method used in the present work was an application of the "stick-slip" method of Bowden and Leben (1939), which was originally developed for the study of metallic friction. Stick-slips are familiar phenomena occurring between sliding surfaces when the motion of one of the surfaces is constrained by an elastic restoring force. During the motion the two surfaces stick together until the restoring force exceeds the frictional force, when a slip occurs. The surfaces stick again and the process is repeated indefinitely.

The apparatus used for these experiments was that of the Lubricants and Bearings Section of the Council for Scientific and Industrial Research, Melbourne, which was modified to develop stick-slips between a single wool fibre and a second surface, usually a piece of horn, with small loads of less than 1 g. Horn was chosen as the second surface because chemically it is related to wool and possesses mechanical properties which in the particular apparatus used made it more convenient to handle than a second wool fibre. Some measurements, however, made by rubbing wool fibre on wool fibre gave essentially the same results.

The fibre was mounted in a reversible bow carried on the end of a lever by means of which the fibre was pressed against the second surface (horn or fibre) with the required load. The horn was fixed to the end of a piece of clock spring. On moving the fibre forward against the horn by means of a hydraulically driven piston (velocity 0·01 cm. per sec.) the horn and fibre stick, and the frictional force is measured by the deflection of the clock spring. This deflection was recorded photographically by a moving film camera. The inertia of the deflecting system was small and its frequency high enough to enable it to follow readily the fluctuations in friction.

This set-up yielded stick-slips which superficially resembled those recorded by Bowden and Leben (1939) using metallic surfaces. The with-scale (tipward) motion and anti-scale (rootward) motion were clearly distinguished on dry normal wool fibres by the magnitude and character of the stick-slips recorded in the two directions. Fig. 1(a) and (b) are reproductions of typical records.

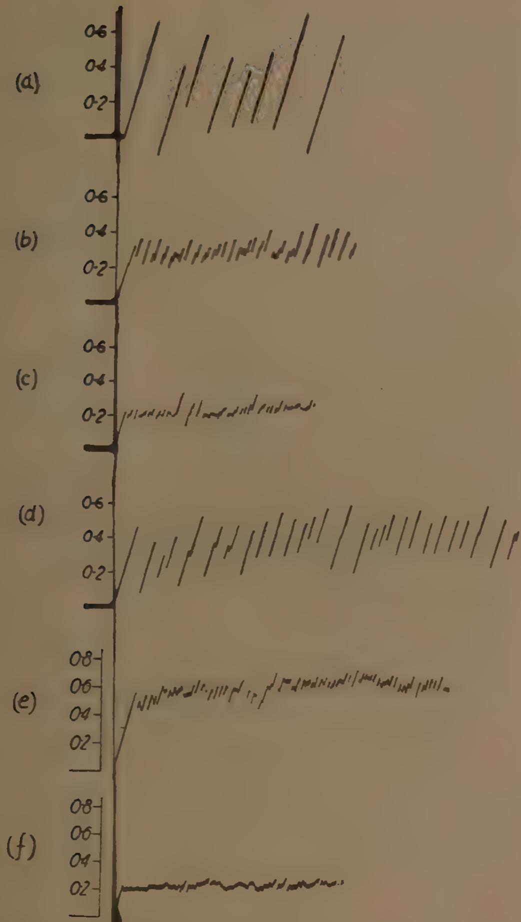

Fig. 1.—Records of "stick-slip" phenomena developed by rubbing wool fibre on horn. The ordinates are the coefficient of friction (μ), and the abscissae time. The lines reproduced are the "stick", during which the frictional force steadily increases until the "slip" occurs. The lighter line in the original record joining the maximum of one stick to the beginning of the next, i.e., the "slip", has been lost in reproduction. The ordinates of the upper ends of the sloping "stick" lines are individual readings of the coefficient of friction (μ).

(*a*) Air-dry wool rubbed in the tip to root direction.
(*b*) Air-dry wool rubbed in the root to tip direction.
(*c*) Wool wet with aqueous solution pH 4 rubbed towards the tip.
(*d*) Wool wet with aqueous solution pH 4 rubbed towards the root.
(*e*) Wool wet with aqueous solution pH 1·3 rubbed towards the root.
(*f*) Wool wet with aqueous solution pH 1·3 rubbed towards the tip.

The effect of wetting or lubricating the rubbing surfaces was observed simply by dropping the liquid on the horn at the point of contact. The apparatus thus allows such variables as load, wetting by various liquids, pH of wetting liquid, time of wetting, oiling, &c., to be easily studied.

3. Some Frictional Properties of Untreated Wool.

The survey of the frictional properties of wool fibres so far made, for the limited purpose of investigating the mechanism of wool felting, has shown that these properties are complex. As there is no accepted theory of the friction of non-metallic surfaces, no satisfactory explanation can be given of some of the phenomena to be described.

The experiments in which a comparison was made between normal and chemically treated fibres were carried out on fibres drawn from the same staple of wool, which was a coarse strong-wool Merino type (58's), sound and uniform to the eye. These observations are therefore confined to one type of wool, but it is not anticipated that changes produced by the felting-reduction treatments will depend to any substantial degree on the nature of the wool.

The staple was degreased by three changes of warm (40–50°C.) petroleum ether and then washed with an aqueous solution containing about 1 per cent. of a sulphonated oil. It was then washed with several changes of distilled water and air-dried. Finally it was hung in the vapour and condensate from a boiling mixture of acetone and isopropyl ether. The horn was cleaned in the same way. Although this treatment was as thorough as could be applied to a substance such as wool, it is not expected that the surfaces were as contamination-free as the cleanest metal or glass surface can be made by more drastic treatment. The question whether the actual keratin surfaces come in contact when air-dry wool is rubbed on air-dry wool must be left for further work. The air-dry wool used had a water content of 12–14 per cent. and no experiments have been made on wool at lower water content. In the case of wet wool, water will probably have displaced the contamination from the surfaces and the solvated surfaces themselves will be in contact.

Air-dry surfaces of wool on polished horn, or wool on wool cleaned in this way, yield characteristic stick-slips, examples of which are reproduced in Fig. 1 (*a*) and (*b*). The coefficient of friction was calculated from the known load and the average value of the maximum frictional force developed during stick. A length of about 1 cm. of fibre was examined in each run, yielding between 20 and 100 discrete stick-slips. The fibre was reversed by rotating the bow through 180° to obtain the coefficient in the opposite direction. The same portion of the fibre was used for the rootward as for the tipward motion.

The surface of the cylindrical piece of horn was polished to minimize the possibility of the scale edges actually catching the unevenesses of the surface. Occasionally, in the records of motion against the scales, a single large stick, indicating a very high coefficient, was found. In computing the average values for μ_2 (dry wool) such large sticks indicating a frictional force greater than twice the average force have been rejected and the average formed from the more uniform events. This procedure may be open to question. Certainly, were all the stick-slips taken into account, a higher value for μ_2 and for δ would result. Stick slips also develop with motion with the scales, showing that the ratchet-like edges are not necessary for

this phenomenon. Stick-slips between metallic surfaces are attributed by Bowden and Leben to the formation and shearing of metallic junctions. Presumably an analogous adhesion of protuberances occurs in the case of the surfaces of wool as is suggested by Fig.4. The variations in μ produced by wetting the fibre can be attributed to changes in this adhesion. This question will be discussed later.

The frictional difference between rootward and tipward motion is of particular interest for the present study. The "scaliness" coefficient of Speakman, Stott, and Chang (1933) has already been mentioned. In the present work a coefficient of frictional difference $\delta = \frac{\mu_2 - \mu_1}{\mu_2 + \mu_1}$ is used. The general properties of the two coefficients are similar and the later is preferred in the present investigation on account of the large differences between μ_1 and μ_2 with which we are concerned. The reasons for calling δ a coefficent of frictional difference instead of scaliness, have already been given.

On wetting fibre and horn there occur changes which result in an increase in the value of the frictional difference. Generally μ_2 increases and μ_1 decreases, as can be seen from Table 1. The frictional changes occur within a minute after wetting, and thereafter the new values for the coefficients remain unaltered for periods up to 1 hour, even in alkaline solution (pH 10·8).

TABLE 1.—TYPICAL COEFFICIENTS OF FRICTION OF WOOL FIBRE.

Load 0·20g.

μ_1 coefficient for motion towards the fibre tip (with scale).

μ_2 coefficient for motion towards the root (anti-scale).

No.	Type of Fibre.	Rubbing Surfaces.	Conditions.	μ_2	μ_1	δ*
1	Normal ..	Wool on horn	Air-dry ..	0·55	0·33	0·25
2			Wet pH 4 ..	0·66	0·32	0·35
3			Wet pH 10·8	0·66	0·25	0·45
4			Wet pH 1·3	0·72	0·32	0·38
5	Normal ..	Wool on wool	Wet pH 4 ..	0·55	0·25	0·37
6			Wet pH 10·8	0·54	0·20	0·46
7	Chlorine treated †	Wool on horn	Wet pH 4 ..	0.11	0·11	0·00
8			Wet pH 10·8	0.02	0·02	0·00
9			Wet pH 1·3	0·12	0·11	0·04
10	Bromine treated †	Wool on horn	Wet pH 4 ..	0·25	0·21	0·09
11			Wet pH 10·8	0·05	0·05	0·00
12			Wet pH 1·3	0·17	0·15	0·06
13	Alcoholic caustic potash treated †	Wool on horn	Wet pH 4 ..	0·62	0·42	0·20
14			Wet pH 10·8	0·53	0·35	0·20
15			Wet pH 1·3	0·72	0·40	0·28
16	Sulphuryl chloride treated †	Wool on horn	Wet pH 4 ..	0·75	0·65	0·08
17			Wet pH 10·8	0·45	0·42	0·03
18			Wet pH 1·3	0·90	0·81	0·05

* The coefficient of frictional difference (δ) = $\frac{\mu_2 - \mu_1}{\mu_2 + \mu_1}$.

† Shrinkage-reduction treatments as described in text. The chlorine-processed fibres have probably been "over-treated".

Particular interest concerns the values of δ in acid and alkaline solution, since many experiments (Speakman, Stott and Chang, 1933; Mercer, 1942) have shown that the felting rate increases in acid and alkaline solutions. The frictional coefficients were therefore determined at pH 1·3 and pH 10·8, values which are outside the iso-electric range of wool. As can be seen from Table 1, the increase in δ is greater at pH 1·3 and pH 10·8 than it is at pH 4·0, a point within the iso-electric range. This would imply as suggested earlier that the increase in δ with pH contributes to the change in felting rate with pH. Fibre extension and contraction cannot therefore be regarded as the only variables contributing to the form of the relation connecting felting rate and pH, since the variation in δ with pH could also give rise to a similar relation. Probably both variables contribute, but further work is necessary to clarify this point.

4. Frictional Changes Produced by Shrinkage-Reduction Treatments.

The earlier commercial attempts to give a non-felting finish to wool by means of chlorine usually removed or badly damaged the cuticular scales of the fibres. Such treatment of course confers the desired property on the wool, but it was soon realized to be far more drastic than was necessary. Present day practice seems to recognize that ideally the chemical alteration is to be confined to the surfaces of the cuticular scales, leaving them visibly intact. Attempts to develop shrinkage-reduction processes of this type must solve two problems: firstly the selection of a suitable keratinolytic reagent, and secondly the devising of means to bring this reagent in contact with the fibre while confining its action to the superficial layers of the fibre. Potentially, any substance that attacks keratin seems capable of yielding a shrinkage-reduction process. Practically, however, the choice is limited by economic conditions rather than chemical. Methods of confining the attack to the surface of the fibre include: limiting the amount of reagent in the treatment liquor, limiting the time of treatment, dissolving the reagent in an inert solvent to dilute the action, and if the reagent does not swell the fibre this fact also limits the action by preventing the larger molecules of the reagent from entering the fibre pores. If the compound will gasify, it may be applied in that form. Temperature of the treatment and the water content of the wool must be carefully controlled.

With the great range of possible treatments it was necessary to restrict the choice for the present experiments. The action in all cases is progressive, and a variety of frictional effects may be expected to result merely from varying the degree of the treatment. The treatments chosen are the three most commonly used: the wet chlorine and bromine, the drisol (sulphuryl chloride), and the alcoholic alkali (Freney-Lipson); the degree of treatment is that usual in commercial practice. The reduction in felting rate is not the same in each instance and this was expected to show up in the frictional measurements.

Details of the actual treatments applied are as follows:—

(i) *Chlorine Treatment.*

A small staple of the degreased and cleaned sample was added to some lengths of yarn and the whole treated together in an aqueous hypochlorite-hydrochloric acid bath containing available chlorine equivalent to 4 per cent of the weight of the wool treated; pH = 2, temperature = 25°C. After treatment the wool was washed in 2 per cent. sodium bisulphite solution. The sample was then washed with water overnight, dried, and degreased by a brief exposure to the condensate from the boiling acetone—isopropyl ether mixture.

(ii) *Bromine Treatment.*

The action of bromine is similar to but more easily controlled than that of chlorine. Treatment bath: 4 per cent. bromine in 0·01N HCl. A staple and yarn treated with 25 times the weight of solution at 25°C. The action was terminated at the end of three minutes, and the excess bromine was removed with sodium bisulphite. The sample was cleaned as described above.

(iii) *Sulphuryl Chloride.*

Treatment was carried out in a 2 per cent. solution in white spirit. Ratio wool: liquor 7 : 1. Regain of wool approximately 15 per cent. Treatment ½ hour at 15°C. rising to 25°C. The staple was washed after treatment with several changes of petroleum ether, immersed in distilled water, and brought to neutrality by cautious addition of 0·02N alkali. This procedure was adopted to avoid exposing the fibres to solutions of pH greater than 7 before the frictional properties at that pH had been determined.

(iv) *Caustic Alkali Process.*

This process was developed in this country by Freney and Lipson (1940) and in England by Hall and Wood (Hall, 1939; Hall and Wood, 1939). The caustic alkalis in aqueous solution rapidly destroy wool and in practice the solvent used is usually alcohol with a small percentage of water.

Seven per cent. caustic potash and 5 per cent. water were dissolved in alcohol The wool was treated at 25°C. for 90 seconds. The alkali was neutralized in 7 per cent. sulphuric acid in alcohol, washed well with water and re-cleaned as above.

The same experimental procedure was followed to determine the three coefficients μ_1, μ_2 and δ as for normal wool. Table 1 summarizes the results obtained, and enables a comparison to be made with those of untreated wool. Figs. 2 and 3 reproduce actual records obtained from treated fibres.

Turning attention first to the frictional difference (δ) it is clear that in all cases the chemical treatment has reduced the value of δ substantially. This is the primary cause of the reduction in felting rate produced by the treatments. The reduction in δ is least in the case of the alcoholic caustic potash treatment as would be expected from the experiments on shrinkage. The several treatments appear to produce the reduction in δ in alkaline media in different ways. The value of δ of greatest interest from the point of view of unshrinkable treatments is that found in alkaline solutions since normal washing solutions usually have a pH between 10 and 11.

Table 1 shows that the values of μ_1 and μ_2 are greatly altered by the chemical treatments, but until more is known of the nature of friction between non-metallic surfaces, we cannot have a complete account of the reason for the changes. Nevertheless the frictional behaviour of

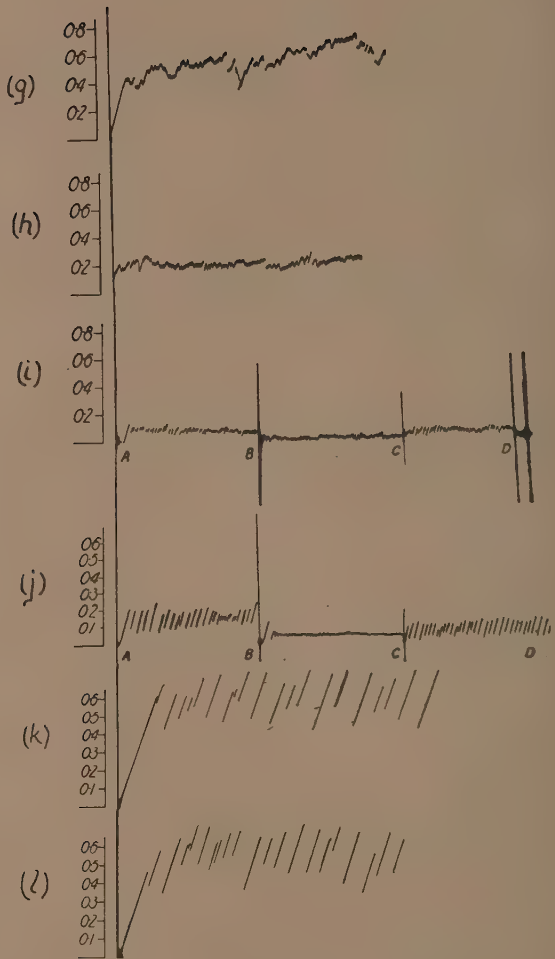

FIG. 2.

(*g*) Wool wet with solution pH 10·8 rubbed towards the root.

(*h*) Wool wet with solution pH 10·8 rubbed towards the tip.

(*i*) Fibre treated with chlorine rubbed towards the root. Section AB wet with water, BC wet with alkaline solution, CD wet with acid solution.

(*j*) Fibre treated with bromine. Details as for Fig. (*i*).

(*k*) Fibre treated with sulphuryl chloride wet with solution pH 4 and rubbed towards root.

(*l*) Fibre treated with sulphuryl chloride wet with solution pH 4 and rubbed towards tip.

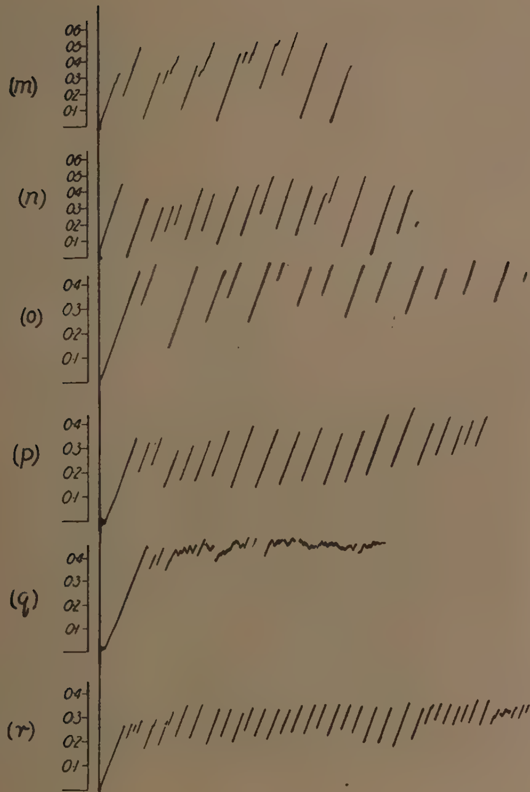

FIG. 3.

(*m*) Fibre treated with sulphuryl chloride, wet with solution pH 10·8 and rubbed towards the root.

(*n*) Fibre treated with sulphuryl chloride, wet with solution pH 10·8 and rubbed towards the tip.

(*o*) Fibre treated with alcoholic caustic potash wet with solution pH 4 and rubbed towards the root.

(*p*) Fibre treated with alcoholic caustic potash wet with solution pH 4 and rubbed towards the tip.

(*q*) Fibre treated with alcoholic caustic potash, wet with solution pH 10·8 and rubbed towards the root.

(*r*) Fibre treated with alcoholic caustic potash, wet with solution pH 10·8 and rubbed towards the tip.

the fibres should be of help in classifying the types of attack. Speakman and Neish (1945) expressed the view that a common chemical mechanism, viz. fission of disulphide links, underlies the action of all effective reagents. The fission of the disulphide bonds is thought to result in the formation of a gelatinous degradation product over and under the scales.

Both chlorine and bromine rapidly attack wool giving rise in acid solutions to the curious blistering of the cuticle known as Allworden's reaction. For an account of the attempts to explain this reaction reference may be made to Frölich, Spottel, and Tanzer (1929). When the fibre is subsequently wet with alkali, the bubbles collapse and the degraded protein apparently readily altered by alkali, appears as a jelly enveloping the fibre. Speakman and Goodings (1926) considered that the gelatinous layer rendered the scales inoperative leading to a non-felting wool. This gelatinous layer between the two relatively hard surfaces of the unmodified keratin would be an effective lubricant and its presence accounts for the low values of μ found (0·02). When acidified the degraded layer loses its jelly-like character and at the same time the friction rises. This behaviour may be seen in the records reproduced as Fig. 2 (*i*) and (*j*). The change is reversible several times, but the degraded layer is gradually scraped off, the friction in alkaline solution thereafter rising.

Alkali treatment and sulphuryl chloride treatment lead to different effects. The coefficients μ_1 and μ_2 are increased in alkaline solution to high values of the order of 0·6—0·9. This is probably due to a surface of altered protein, different from that produced by the action of chlorine.

That the end results of alkali attack and sulphuryl chloride are, however, distinct from each other is shown by the frictional measurements in acid solution. μ_2 and μ_1 for sulphuryl chloride treated wools are high in acid media and δ is low. Wool treated by this process is therefore unshrinkable in acid media. The value of δ for alkali-treated wools in acid solution more nearly resembles normal wool and, as Mercer (1942) has shown, felting properties in acid solution are similar also.

We conclude, therefore, that even if the initial attack is at the disulphide links, this reaction must be followed by others peculiar to the several reagents and leading to different end products.

The values of μ given in Table 1 apply only to fibres treated as described above. Somewhat different results can be obtained by different degrees of treatment. For instance "overtreatment" by sulphuryl chloride leads to lower values of μ_2 and μ_1 suggesting a gelatinous layer in this case also, although none can be seen. It appears from the results of Whewell, Rigelhaupt, and Selim (1944) that the treatment using chlorine dissolved in carbon tetrachloride is different from the treatment used here, and resembles more that of sulphuryl chloride.

5. The Physical Basis of Fibre Friction.

The discussion of these results would not be complete without a comment on the physical basis of the friction between fibres. The outstanding problem is the explanation of the frictional difference. Is the currently accepted theory, that frictional difference arises from the mechanical ratchet effect of the imbricated cuticular scales, capable of explaining the phenomena described in this paper? The most important and obvious evidence for this theory is the microscopic appearance of the fibre. The variation of δ with pH indicates, however, that the simple picture is not enough. This complication may be accounted for by postulating that the angle the scales make with the

fibre axis is influenced by pH., e.g. by the swelling of a sub-cuticular layer. The changes effected by shrinkage-reduction reagents would then be related to an attack on this sub-cuticular swelling agent and/or to the production of a thin layer of degraded protein sealing the scales down. Such an elaboration of the theory would still be unsatisfactory, since the increase in alkaline media results mainly from a fall in μ_1 (i.e. the with-scale coefficient).

When we analyse more closely the nature of the contact between fibres and other solid surfaces, further difficulties arise, some of which have already been pointed out by Martin (1944). The ratchet effect of the scales can only be manifest if (1) there exist unevenesses of similar order of size on the second surface to allow of interlocking, or (2) a ploughing of the scale edges into the second surface can occur. But as Martin reported, wet fibres exhibit directional friction against a glass surface. The present author has observed the marked migratory power of wet fibres held between two oscillating optically flat pieces of glass. The coefficients determined in this paper are those of wool on a polished cylinder of horn. These surfaces present no unevenesses of the order of cuticular scale edges, nor in the case of glass can ploughing occur.

On the other hand a ratchet surface can exist without conferring a marked directional effect. For instance, appropriate treatment with sulphuryl or alcoholic caustic potash leaves the fibre microscopically unaltered and yet the value of δ is much reduced. Moreover, Martin has by a casting process produced surfaces identical in shape with fibres but which were devoid of a directional effect.

If we bear in mind the general principle, well established experimentally by Bowden and Tabor (1939) in the case of metallic surfaces, that contact of solid surfaces can occur only over an extremely small fraction of the apparent area of contact, then it is reasonable to suppose that the friction arises, as with metals, from the formation and shearing of junctions between protuberances of a much smaller order of size than the cuticular scale edges (Fig. 4).

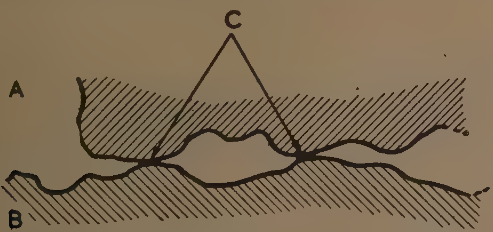

Fig. 4.—Hypothetical form of contact between an epithelial scale edge (A) and a smoother surface such as polished horn (B). Contact can only occur over small areas, e.g. C, and friction arises from the force required to shear the adhesion resulting from contact.

In the case of metals, the magnitude of the frictional force has been well correlated by Bowden and Tabor (1942) and by Ernst and Merchant (1940) with the shear strength of the metals participating in the junction, justifying the view that the protuberances are literally welded together.

With keratin and some other non-metallic surfaces, welding in this way is not possible, but a cohesion between points of contact may be expected to result from chemical bonding as suggested by Fig. 4. That the coefficients of friction are sensitive to pH indicates that among the participating bonds are the acid and basic groups of the protein. The higher coefficients (0·7–0·9) exhibited under some conditions by fibres treated for instance with sulphuryl chloride may be related to the increased number of reactive surface groups whose existence is shown independently by the greater wettability of the surface of these fibres and by marked reaction with Rimington's test (free tyrosine groups) (1930).

The explanation of the frictional difference is more difficult. We may assume that the adhesions are of such a nature that they are more easily sheared by motion in one direction than in the other. The shear may occur either in the surface of contact, in which case we would have to assume the existence of unsymmetrical bonds perhaps after the manner postulated by Martin (1944), or in the keratin itself. The action of shrinkage reduction process would be the reduction of the frictional difference by destroying the property of the surface which leads to the formation of the unsymmetrical adhesions. There is insufficient experimental evidence to discuss the question.

The action of shrinkage reduction process would be the reduction of the frictional difference by destroying the property of the surface which leads to the formation of the unsymmetrical adhesions.

6. Acknowledgments.

The author wishes to thank Dr. F. P. Bowden and Dr. D. Tabor for permission to use the facilities of the Section of Lubricants and Bearings, Melbourne, and Dr. Tabor for his help in devising the apparatus and in discussion.

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Further Observations on the Stability of Lime-Sulphur During Dipping or Spraying Sheep.

*By J. L. Hill, A.S.T.C.**

Summary.

1. Detailed analyses are given showing the change in chemical composition of lime-sulphur fluid during the progress of four dipping trials, in which lime-sulphur was used at an initial concentration of approximately 1 per cent. weight/volume polysulphide sulphur. One trial was conducted with a swim dip and the other three with power-sprays.

2. When using the swim dip the polysulphide sulphur concentration and the polysulphide ratio never fell below effective strengths during the period of dipping. The decrease in polysulphide sulphur concentration was negligible when the dip was allowed to remain undisturbed over night.

3. Lime-sulphur may be used as a dipping agent in power-spray units provided the volume of fluid in circulation never falls below 200 gallons.

4. Amounts of wetting agents found suitable for use with lime-sulphur dips and sprays are given.

5. When sheep that were dipped immediately off-shears were examined thirteen days later, shear cuts were inflamed and suppurating and some of them were fly-blown. Sheep therefore should not be dipped in lime-sulphur immediately off-shears, but preferably seven to ten days later when the shear wounds have commenced to heal.

1. Introduction.

Following the identification of the sheep itch mite, *Psorergates ovis,* as the cause of a skin disease of sheep by Carter (1941), Graham (1943) published the results of a field trial showing that a lime-sulphur solution containing more than 0·4 per cent. w/v of polysulphide sulphur was effective in controlling this mite.

Although calcium polysulphide preparations have been used extensively as a sheep dip in South Africa and U.S.A., and have always been regarded as rather unstable, little information is available regarding their stability. Hambrock *et al.* (1934) have published the only comprehensive information. They showed that, when using either home-made or proprietary concentrates, decomposition may occur by decrease in polysulphide concentration or by changes in the nature of the polysulphides. In one instance they reported a used dip containing $CaS_{1.3}$ which would contain appreciable quantities of HS ions and hence be liable to damage the wool fibre.

In view of the successful control of *Psorergates ovis* by lime-sulphur (Graham, loc. cit.) a more extensive investigation of the chemical changes associated with the use of this product as a dip or spray, was desirable.

Hill (1942) analysed samples of dip taken at various stages during the field trial conducted by Graham. It was found that the polysulphide concentration remained within effective limits during dipping and that the polysulphide ratio never fell below 4·3. A swim dip was used in this instance, however, and it was considered that the decomposition of calcium polysulphides might be much greater when a power-spray was used, owing to increased aeration of the fluid. Additional experi-

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ments to study this have therefore been carried out. This paper is a report of three trials with power-sprays and of a further trial with a swim dip.

2. Preparation of Lime-Sulphur Fluid.

In all four trials the fluid was prepared by adding five gallons of a proprietary concentrate containing approximately 21 per cent. polysulphide sulphur, to 100 gallons of water. The resultant fluid contained approximately 1·0 per cent. of polysulphide sulphur. A proprietary concentrate was preferred because it is a standardized product whereas in home-made concentrates the percentage of polysulphide sulphur has been found to vary considerably. In each trial rain-water was used in the preparation of the fluid.

For the swim dip, 6·0 fl. oz. of a wetting agent, "Agral L," per 100 gallons of fluid were used for the initial fill, and 3·0 fl. oz. for each of the two subsequent additions of concentrate and water required to re-adjust the volume owing to removal of fluid in the fleeces of the sheep. Thereafter sufficient suint had accumulated to render the further addition of wetting agent unnecessary. In the first trial with a power-spray, in which the sheep were carrying approximately a nine-weeks' growth of fleece which was dense with a very "blocky" tip, 6 oz. of wetting agent per 100 gallons were used and the result was fairly satisfactory. In the second trial in which the sheep were a fortnight off-shears, 6 fl. oz. of wetting agent per 100 gallons were also found sufficient for the initial charge. Suint was leached out so quickly that there was no need to add further wetting agent.

In one of the above trials "Wetsit" was substituted for "Agral L" and was found to be satisfactory.

3. Sampling Technique and Chemical Analyses.

Samples were taken at intervals during dipping or spraying and Chapin's (1915) field test for sulphide-sulphur concentration was applied. South African workers (Wilken-Jorden, 1932) do not favour the use of Chapin's method because they found that when the polysulphide ratio falls to low levels results obtained by it can be misleading. In our work, however, we found that the polysulphide ratio has remained fairly constant at approximately 4·5 and under such conditions Chapin's field test will furnish a reliable estimate of changes in polysulphide concentration.

After testing by Chapin's method the remainder of the sample was poured into a black-painted bottle which was completely wrapped in brown paper, and paraffin oil or benzol was added to the sample to prevent access of air. On receipt at the laboratory the samples were immediately analysed by using the methods of the Association of Official Agricultural Chemists (1940).

4. Results.

Trial 1: Swim Dip.

Every morning before the commencement of dipping, a sample was taken after thorough agitation of the dip-fluid (sample 1-). The volume of fluid was then adjusted by the addition of concentrate and water, and another sample taken (sample 2-). At the conclusion of each day's dipping a further sample was taken (usually sample 3-).

Table 1 summarizes the results. No appreciable loss in strength occurred when the dip was left undisturbed over a night or a week-end. Over-night loss in polysulphide sulphur was less than 0·02 per cent. in every case. In this trial it was never necessary to add extra lime-sulphur concentrate, because a reasonable dipping strength was always maintained even though the trial occupied a fortnight. Sampling errors would explain the irregular results obtained with Samples 2C and 1H. Care should be exercised in interpreting results of samples with pH less than 9·0, as it is possible that bacteriological decomposition may occur before analyses can be completed. Reference to bacterial decomposition is made later.

TABLE 1.—ANALYSES OF SAMPLES FROM A SWIM DIP.—TRIAL 1.

Date (1943).	Number of Sheep.	Sample Number.	Sulphide Sulphur Percentage w/v.		Monosulphide Sulphur Percentage w/v.	Polysulphide Sulphur Percentage w/v.	Polysulphide Ratio.	Thiosulphate Sulphur Percentage w/v.	Calcium Percentage w/v.	pH.	Additions to Bath.		Volume of Dip before Addition.
			Field Test.	A.O.A.C. Method.							Water (gallons).	Lime-Sulphur Concentrate (gals.)	
26/10	nil	2	1·2	1·126	0·239	0·887	4·70	..	0·492	10·4	1550	81	nil
	304	3	..	1·120	0·239	0·881	4·68	0·258	0·481	10·0	..	..	..
28/10	304	1A	1·1	1·115	0·239	0·876	4·66	0·265	0·480	10·1	80	4	1400
	1379	2A	0·9	0·969	0·205	0·764	4·72	0·302	0·452	9·8	..	..	..
29/10	1379	1B	..	0·956	0·204	0·752	4·69	0·310	0·439	9·7	..	..	..
	1379	2B	0·9	0·965	0·206	0·759	4·69	0·274	0·440	9·8	375	19	1125
	2216	3B	0·7	0·856	0·180	0·676	4·75	0·302	0·392	9·6	..	..	..
30/10	2216	1C	..	0·841	0·180	0·661	4·67	0·310	0·399	9·5	..	..	..
	2216	2C	0·8	0·820	0·176	0·644	4·65	0·282	0·400	9·6	290	15	1210*
	2644	3C	0·8	0·820	0·195	0·625	4·20	0·318	0·400	9·4	..	..	..
1/11	2644	1D	..	0·800	0·187	0·613	4·28	0·318	0·400	9·4	..	..	..
	2644	2D	0·8	0·844	0·188	0·656	4·49	0·308	0·421	9·7	160	8	1340
	3048	3D	0·75	0·788	0·175	0·613	4·50	0·326	0·394	9·4	..	..	..
2/11	3048	1E	..	0·768	0·171	0·597	4·49	0·320	0·388	9·3	..	..	..
	3048	2E	0·8	0·870	0·189	0·681	4·60	0·316	0·434	9·5	270	14	1230
	3739	3E	0·7	0·711	0·160	0·551	4·45	0·368	0·413	9·1	..	..	..
3/11	3739	1F	..	0·636	0·139	0·497	4·57	0·338	0·400	8·3	40	..	1320
	3739	2F	0·75	..	..	..	..	..	..	..	140	11	1360
4/11	3739	2G	..	0·796	0·167	0·629	4·76	0·387	0·470	9·5	235	11	1360
	4355	3G	..	0·702	0·151	0·551	4·65	0·409	0·412	9·4	..	..	..
6/11	4355	1H	..	0·731	0·163	0·568	4·49	0·394	0·425	8·7	40	..	1290†
	4355	2H	0·75	0·808	0·176	0·632	4·59	0·368	0·440	9·5	170	11	1330
	5205	3H	..	0·676	0·160	0·516	4·23	0·402	0·372	8·5	..	..	..
	5205	4H	..	0·755	0·168	0·587	4·50	0·368	0·410	8·8	250	12	1020
	5627	5H	..	0·615	0·149	0·466	4·13	0·388	0·369	8·4	..	..	..
8/11	5627	1J	..	..	..	..	..	0·378	0·368	8·5	..	..	..
	5627	2J	0·75	0·615	..	..	..	0·329	0·396	8·9	335	18	1145
	6560	3J	..	0·543	0·130	0·413	4·18	0·361	0·382	8·3	..	..	1150
	6560	4J	..	..	0·115	..	..	0·410	0·320	8·2	..	..	..‡

* Poor sample. Emptied draining sump. Loss of 100 gallons.——————† Poor sample.
‡ Drainings from draining pens.

Trial 2: Power Spray.

Trial 2 was carried out with a power-spray unit having a sump of 400 gallons capacity and driven by a 1½-in. pump delivering 70 gallons of spray dip per minute at a pressure of 50 feet head of water. There was approximately 100 gallons of fluid in circulation at any one moment. Its capacity was 30 sheep. Unfortunately, these sheep carried approximately nine weeks' wool and, as a result, each removed more than a gallon of fluid. Consequently frequent re-adjustments of the volume in the sump had to be made and hence the strength was, at the same

time, increased. The sheep were sprayed for eight minutes, three minutes with the top sprays, then two minutes with the bottom sprays and finally three minutes with the top sprays again. The results in Table 2 show that towards the conclusion of spraying on the second day the fall in polysulphide concentration was very rapid. This was because the volume of fluid was allowed to decrease so that the sump would be almost empty at the conclusion of spraying. On the first day, however, when the volume of fluid in the sump was carefully regulated, although there was an appreciable fall in polysulphide content (Samples 3-5), it did not fall below effective levels. Hill (loc. cit.) has shown that, in a swim dip, decomposition becomes progressively greater as dipping proceeds. The sudden fall in polysulphide concentration in this trial was probably due to this factor as well as to a smaller volume of fluid being re-circulated more quickly.

TABLE 2.—ANALYSES OF SAMPLES FROM TRIAL 2: POWER-SPRAY.

Date (1944).	Number of Sheep.	Sample Number.	Sulphide Sulphur Percentage w/v.		Monosulphide Sulphur Percentage w/v.	Polysulphide Sulphur Percentage w/v.	Polysulphide Ratio.	Thiosulphate Sulphur Percentage w/v.	Sulphite and Sulphate Sulphur Percentage w/v.	Calcium Percentage w/v.	Additions to Sump.		Volume in Sump before Addition.
			Field Test.	A.O.A.C. Method.							Water (Gallons).	Lime-Sulphur Concentrate (gals.).	
19/1	Nil	1	1·35	1·220	0·276	0·944	4·41	0·067	negl.	0·394	400	20	Nil
	160	2	1·2	1·009	0·222	0·787	4·55	0·130	negl.	0·370	..	..	..
	232	3	1·05	0·951	0·216	0·735	4·40	0·130	0·002	0·326	180	9	220
	386	4	0·9	0·835	0·185	0·650	4·51	0·169	0·004	0·316	..	..	..
	548	5	0·7	0·619	0·133	0·486	4·65	0·248	0·014	0·308	..	..	..
	548	6	1·45	1·393	0·312	1·081	4·46	0·185	0·008	0·520	180	17	200
	701	7	1·4	1·286	0·282	1·004	4·56	0·236	0·016	0·500	..	..	..
	809	8	1·15	1·103	0·228	0·875	4·84	0·316	0·028	0·435	..	..	..
20/1	809	1A	1·15	1·119	0·239	0·880	4·68	0·175	0·006	0·405	200	10	200
	931	2A	1·05	0·985	0·207	0·778	4·76	0·210	0·012	0·388	..	..	..
	1040	3A	0·95	0·837	0·187	0·650	4·48	0·244	0·019	0·373	..	..	..
	1075	4A	1·05	1·039	0·234	0·805	4·44	0·166	0·009	0·398	200	10	..
	1182	5A	1·05	0·916	0·198	0·718	4·63	0·196	0·013	0·361	..	..	..
	1182	6A	..	0·805	0·180	0·625	4·47	0·206	0·028	0·348	..	..	..*
	1182	7A	..	0·606	0·139	0·467	4·36	0·242	0·026	0·300	..	..	..†
	1319	8A	0·95	0·754	0·175	0·579	4·31	0·213	0·018	0·338	..	..	..
	1443	9A	..	0·426	0·097	0·329	4·39	0·298	0·024	0·308	..	..	..
	1488	10A	..	0·314	0·064	0·250	4·90	0·338	0·029	0·250	..	..	100

* Drainings from spraying pen.——† Drainings from draining pens.

Trial 3: Power Spray.

This power-spray unit had a sump of approximately 550 gallons capacity and was driven by a $2\frac{1}{2}$-in. pump developing 170 gallons per minute at a pressure of 60 feet head of water. Sixty sheep were sprayed together. This time the top sprays were used for three minutes and the bottom sprays for two minutes. The sheep were about a fortnight off-shears and each removed approximately half a gallon of fluid. On the first day the fluid was prepared as previously described but on the second and third days sucrose was added at the rate of 0·5 lb. per gallon. Sucrose has been added in the commercial preparation of dry lime-sulphur because it is said to act as a stabilizing agent. However, in the present investigation its use did not prevent

oxidation of the calcium polysulphides. Moreover, when an attempt was made to analyse those samples containing sucrose, a week after sampling, it was found that most of them had partially decomposed.

Chapin's Field Test proved quite accurate under the conditions of this investigation (see Tables 1 and 2). If, then, we accept it as a guide, it can be seen in Table 3 that so long as the volume in the sump never falls below 200 gallons, satisfactory dipping strength can be maintained. On the first day the volume was allowed to fall below this level and the dip then decomposed very quickly (samples 9, 10, and 11). Sample 7 was taken when the volume was about 200 gallons and is below the minimum effective strength of 0·4 per cent. w/v polysulphide sulphur. However, the initial concentration (Sample 1) was only 0·646 per cent. w/v polysulphide sulphur, whereas in normal circumstances it would be approximately 1·0 per cent. Had this been the case here the strength would presumably have been greater than 0·4 per cent. when the volume in the sump had fallen to 200 gallons. At this stage the volume would be re-adjusted and hence the strength of the dip would be automatically increased.

TABLE 3.—ANALYSES OF SAMPLES FROM TRIAL 3: POWER-SPRAY.

Date (1943).	Number of Sheep.	Sample Number.	Sulphide Sulphur Percentage w/v.		Monosulphide Sulphur Percentage w/v.	Polysulphide Sulphur Percentage w/v.	Polysulphide Ratio.	Thiosulphate Sulphur Percentage w/v.	Calcium Percentage w/v.	pH.	Additions to Sump.		Volume in Sump before Addition (gallons).
			Field Test.	A.O.A.C. Method.							Water (gallons).	Lime-Sulphur Concentrate (gals.).	
30/11	nil	1	0·85	0·810	0·164	0·646	4·95	0·186	0·329	10·5	500	25	nil
	323	2	0·7	0·665	0·135	0·530	4·93	0·217	0·306	9·9	..	..	..
	430	3	0·72	0·691	0·137	0·554	5·05	0·222	0·309	9·8	175	8	325
	836	4	0·6	0·531	0·115	0·416	4·62	0·260	0·309	9·4	..	..	..
	974	5	0·8	0·711	0·145	0·566	4·90	0·348	0·411	9·4	..	6	400
	1131	6	0·6	0·525	0·103	0·422	5·10	0·398	0·392	9·0	..	..	..
	1281	7	0·5	0·340	0·074	0·266	4·60	0·474	0·370	8·6	..	..	..
	1366	8	0·3	0·156	0·033	0·123	4·74	0·535	0·350	7·9	..	..	..
	1366	9	0·4	0·252	0·055	0·197	4·59	0·675	0·414	8·0	..	2	175
	1517	10	..	0·062	0·0136	0·048	4·55	0·695	0·417	7·2	..	..	..
	1565	11	0·1	0·009	..	..	..	0·780	..	6·9	..	..	50
1/12	1565	1A	1·05	0·965	0·192	0·773	5·03	0·107	0·316	10·3	500	25	nil
	1833	2A	0·97	0·940	0·187	0·753	5·02	0·138	0·314	9·5	..	..	..
	1999	3A	0·85	0·826	0·163	0·663	5·07	0·169	0·312	7·8	..	..	300
	2196	4A	0·75	0·695	0·134	0·561	5·19	0·209	0·307	8·1	..	..	..
	2196	5A	0·75	0·678	0·134	0·544	5·05	0·210	0·307	7·0	20	1	260
	2401	6A	..	0·525	..	..	..	0·251	0·288	6·3	..	..	..
	2401	7A	0·9	0·911	0·181	0·730	5·04	0·164	0·326	8·9	260	13	200
	2676	8A	0·85	..	..	..	..	0·210	..	6·9	..	..	..
	2789	9A	0·75	..	..	..	..	0·246	0·329	7·0	..	..	..
	2927	10A	0·65	..	..	..	..	0·334	0·331	6·5	..	..	..
	2987	11A	0·55	..	..	..	..	0·411	0·328	6·0	..	..	200
2/12	2987	1B	0·95	..	..	..	..	0·213	0·345	7·0	330	17	200
	3201	2B	0·85	..	..	..	..	0·228	0·354	7·2	..	..	..
	3389	3B	0·75	..	..	..	..	0·254	0·342	7·2	..	..	..
	3579	4B	0·7	..	..	..	..	0·293	0·350	6·5	..	..	..
	3701	5B	0·6	..	..	..	..	0·360	0·354	6·8	..	..	..
	3867	6B	0·4	..	..	..	..	0·400	0·340	5·8	..	..	..
	3940	7B	0·35	..	..	..	..	0·438	0·330	6·0	..	..	..

Trial 4: Power Spray.

From the data obtained in Trials 2 and 3 it was considered that it would not be difficult for graziers to maintain effective concentration of polysulphide sulphur without the aid of chemical control, provided sufficient care was exercised in maintaining volume.

Table 4 summarizes data obtained from samples forwarded to this laboratory by a grazier who sprayed 2,800 sheep in a power-spray unit similar to that used in Trial 3. Instructions based on results obtained in previous trials were given but no personal supervision was exercised.

TABLE 4.—ANALYSES OF SAMPLES FROM TRIAL 4: POWER-SPRAY.

Date.	Sheep.	Sulphide Sulphur Percentage w/v.	Mono-sulphide Sulphur Percentage w/v.	Poly-sulphide Sulphur Percentage w/v.	Poly-sulphide Ratio.	Thio-sulphate Sulphur Percentage w/v.	Calcium Percentage w/v.
1st day ..	Nil	1·013	0·220	0·793	4·60	0·069	0·316
,, ..	1800	0·630	0·133	0·497	4·74	0·197	0·291
2nd and 3rd days ..			No spraying owing to rain.				
4th day ..	1800	0·909	0·189	0·720	4·80	0·161	0·338
,, ..	2800	0·513	0·110	0·403	4·66	0·212	0·264

The sump was not cleaned out at the end of the first day's spraying. Prior to commencement of spraying on the fourth day the volume of fluid was re-adjusted by the addition of lime-sulphur concentrate and water. The polysulphide sulphur content was still at an effective level at the conclusion of spraying, thus showing the efficiency of maintaining a correct volume of fluid in the sump.

5. Decomposition of Samples.

When the decomposition of samples was suspected some were stored at room temperature and analysed at varying intervals thereafter. Table 5 shows the changes in sulphide-sulphur concentration which occurred during storage.

TABLE 5.—VARIATION IN SULPHIDE-SULPHUR CONCENTRATION WITH AGE OF SAMPLE.

Sample.		Original Analysis.*	Second Analysis.	Third Analysis.	pH.
		%	%	%	
Unused dip—Trial 1		0·905	0·916 (40)†	0·879 (82)†	10·4
Unused dip—Trial 3		0·810	0·780 (15)	0·709 (52)	10·5
Unused dip		1·013	1·033 (47)	0·889 (68)	..
Unused dip containing 5 per cent. Sucrose (1A—Trial 3)		0·965	0·946 (24)	0·900 (49)	10·3
No. 3—Trial 3		0·691	0·684 (13)	..	9·8 (38)
No. 1E—Trial 1		0·768	0·535 (65)	..	9·3 (62)
No. 7—Trial 3		0·340	0·145 (41)	..	8·6 (27)
No. 3J—Trial 1		0·543	0·298 (40)	0·246 (68)	8·3 (55)
No. 4J—Trial 1		0·392	0·203 (40)	0·146 (68)	8·2 (55)
No. 7A—Trial 3	All samples contain 5 per cent. sucrose	0·911	0·214 (40)	..	8·9 (28)
No. 4A—Trial 3		0·695	0·127 (42)	..	8·1 (30)
No. 2B—Trial 3		0·508	0·180 (13)	..	7·2 (28)
No. 1B—Trial 3		0·754	0·216 (13)	0·182 (41)	7·0 (28)
No. 8A—Trial 3		0·242	0·189 (5)	0·154 (40)	6·9 (28)

* About 7–10 days after samples collected.

† Figures in brackets represent days elapsing since original analysis.

In interpreting these results it must be remembered that sulphide sulphur would include any hydrogen sulphide which was present. For instance, the third analyses of the last two samples in Table 5 included some hydrogen-sulphide sulphur. Moreover, while unused samples and used samples with a pH greater than 9·0 were stable enough to allow an analysis to be made before decomposition was apparent, used samples with a pH of less than 9·0 showed rapid decomposition. When the used sample contained sucrose its decomposition was particularly rapid.

Numerous micro-organisms were present in the used samples, as was to be expected, and the presence of sucrose would encourage the growth of some of them. Such bacteria may have liberated acidic substances which decompose the calcium polysulphide since the final pH on complete decomposition was always less than 7·0. This was due to the presence of free H_2S which would have been formed by reaction with calcium monosulphide. The thiosulphate content remained constant.

6. Field Observations.

In Trial 1 all sheep were dipped immediately off-shears with the exception of one mob of stud ewes and lambs. Owing to inclement weather these were returned to their paddock but were dipped four days later. Examination of the first mob to be dipped, thirteen days afterwards, showed that many shear cuts were inflamed and discharging, and some were fly-struck. No strikes were noted on unshorn sheep at this period. It is probable that the high pH of the dip irritated the wounds. The dip fluid, moreover, contained an appreciable quantity of excreta and hence a high bacterial content. Stud ewes and lambs which were dipped four days off-shears were not fly-struck and shear wounds healed normally.

It would appear desirable, therefore, not to use a lime-sulphur dip or spray until a week or ten days after shearing. The interval should not be too great in view of the necessity of thoroughly wetting the skin of the animals, because the difficulty of wetting increases with wool growth. Moreover, the sooner the sheep are dipped and sprayed after shearing the less fluid they take out in their fleeces.

Since wetting to the skin of the sheep is required for destruction of the itch mite, it is of prime importance to examine sheep periodically during dipping or spraying. However, it has been found that a sheep which, immediately on emergence is poorly wetted may be wet to the skin ten minutes later. It is recommended, therefore, that graziers should not examine sheep on emergence from the dip bath or spray-pen, but just prior to release from the draining pens. Should, then, the sheep not be wetted effectively more wetting agent should be added to the dip-fluid.

7. Discussion.

The results of Trial 1 confirm the findings of the previous trial carried out by Graham (loc. cit.) wherein it was found by Hill (loc. cit.) that lime-sulphur was sufficiently stable for use in swim dips. In Trial 1 there seemed to be a consistent decrease in strength of the dip when allowed to stand undisturbed over-night but it was so small as to

be negligible. Samples were forwarded at various periods after dipping but owing to delays in transit they showed too much decomposition and results are not included in this paper. However, it is recommended that a used dip be kept in the dip bath or sump for no longer than a week.

pH results are included in Table 1. These were determined about two months after the samples were taken. Those results which are less than 9·0 are probably slightly low, because of bacterial decomposition. However, they show the general trend to be expected with oxidized lime-sulphur fluids.

Trials 2 and 3 show that lime-sulphur may be used in power-sprays provided the volume of fluid in the sump is maintained at a reasonable level. In Table 2 it is interesting to note the progressively lower concentration of polysulphide sulphur of the fluid in (*a*) the sump, 0·718 per cent., (*b*) the dip-fluid returning to the sump from the spraying enclosure, 0·625 per cent., and (*c*) the drainings from the draining pens after the sheep had been enclosed for two minutes, 0·467 per cent. Calcium sulphite and calcium sulphate determinations have been included in Table 2. It will be seen that their concentration increases with the oxidation of the bath.

In Table 3 some analyses of samples below 7A were omitted because the low pH suggested that they had partially decomposed and their sulphide-sulphur content was not in reasonable agreement with the field test.

It is now thought that the loss in strength on preparing the dip, reported in an earlier paper (Hill, loc. cit.), was due to a variation in the polysulphide content of lime-sulphur concentrates. It has been shown (Anon. 1936-37) that when full drums of lime-sulphur concentrate were allowed to stand for ten months, a fall of approximately 10 per cent. polysulphide sulphur occurred. Even in two months there was a decrease of from 5 to 10 per cent. It will be noted that in Trials 1 and 3 the concentrate used was not of normal strength, i.e., 21 per cent. w/v polysulphide sulphur.

8. Acknowledgments.

I would like to express my appreciation for the help and co-operation of four graziers, without which this experiment could not have been conducted.

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Reactions of Tasmanian Bismark and Brownell Potatoes to the Commoner Virus Diseases.

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Summary.

A description, designed for use by inspectors and field workers, is given of the symptoms on Bismark and Brownell potato of the mosaic viruses X, A, and Y, and of leaf roll. Bismark is unusually resistant to leaf roll and is susceptible to the disease "crinkle", due to the combination of viruses X and A. All Brownell plants carry virus X, but are field immune to virus A. Brownell is susceptible to leaf roll, which is the main cause of degeneration in this variety.

1. Introduction.

The varieties Brownell and Silverskin Bismark have for years been the basis of the potato export trade from Tasmania to the Australian mainland. Their good quality and natural resistance to mechanical injury and transport rots have ensured them a ready market. In the years leading up to 1927, virus diseases were threatening to destroy their productive capacity, but, by energetic action, officers of the Tasmanian Department of Agriculture obtained and multiplied high-quality foundation stocks of both varieties, which have provided the basis for a successful certification scheme. In 1937, the C.S.I.R., at the invitation of the Tasmanian Department of Agriculture, began a co-operative study of the virus diseases affecting these two varieties. The work has been continued intermittently up to the present time. Some of the results, and knowledge accumulated by observations in the field are summarized here.

2. Nomenclature of the Two Varieties.

The Tasmanian Brownell is apparently the same variety as "Adirondack", which is of American origin. Adirondack was introduced into New South Wales about the beginning of the century. It became widely known as "Satisfaction", a name listed as a synonym by Salaman (1926). At that time Brownell's Beauty was a popular variety, and because of some similarity in the colour and form of the tubers, the variety Adirondack was often marketed as Brownell. It was introduced into Tasmania under that name about 1907. Having a greater resistance to Irish blight than the extremely susceptible and then universally grown Redskin (Brown's River or Circular Head) it became the principal maincrop variety of Tasmania. In time it became known as the Tasmanian Brownell. Adirondack is still grown in parts of New South Wales as Satisfaction.

The history of the Bismark in Tasmania, as far as it is known, is given by Oldaker (1937). The only variety of the same name mentioned in the literature available to the authors is Cimbal's "Fürst Bismark", a late red variety (Rathlef, 1932). The original Tasmanian

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Bismark, first known as "Prince Bismarck", was distinctly different and probably an older variety. The modern Silverskin Bismark is an improved strain of Prince Bismarck, or it has arisen as a chance seedling of the parent variety. It is generally assumed to be an improved strain because growers reported that tubers of the Silverskin type had occasionally appeared in stocks of Bismark for many years before they were selected and multiplied. However, the possibility that it arose as a seedling cannot be ignored, as Bismark has always produced a profusion of fertile seed, and crops were often planted on the same ground in successive seasons.

There is slight circumstantial evidence that it may actually be a seedling variety. Before 1937, a proportion of Silverskin Bismarks in commercial crops was free from both virus X and virus A, but these viruses were spreading rapidly. Samples of the older type of Bismark tested were saturated with a mixture of virus X and virus A. A mutant strain of the older Bismark would probably have carried the same viruses as the parent stock, but a clone developing from a seedling would initially be virus-free. Unless it were infected at a very early stage in its multiplication, it would contain virus-free plants for a number of years, as did the Silverskin Bismark.

There is some genetical evidence that the Silverskin might have arisen by self-fertilization of the parent variety. It appears less heterozygous than most commercial varieties of potato: selfed seed from Silverskin Bismark plants gives rise to an exceptionally homogeneous progeny*. There is, of course, the possibility that the development of clones from selfed seed occurred at an earlier stage in the development of the modern Bismark. Seedlings of the older Bismark have not been grown and tested for homogeneity in comparison with the Silverskin, so the evidence is incomplete.

3. Field Appearance of Diseased Plants.

The earlier descriptions of the diseases attacking Silverskin Bismark and Brownell were made before the viruses causing them had been identified. The principal virus diseases were described as "crinkle" of Bismark and "mild" or "common mosaic" of Brownell (Oldaker and Dowson, 1929). The "crinkle" of Bismark was unaccompanied by mottle. It was also named at different times "rugose mosaic" and "curly dwarf", possibly because an attempt was being made to describe more than one mosaic disease by a single name that did not fit them all.

The "mild mosaic" of Brownell was described as follows:—"The leaves are mottled with light-green to yellow areas, producing, when seen at a little distance, a decided yellow appearance of the whole plant. Infected plants are smaller than healthy ones, and the tubers produced are reduced in size."

Field inspections over a period of years revealed complications which disturbed the simple principle of one variety, one characteristic virus disease. More than a single set of symptoms appeared on diseased

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plants of each variety, and these symptoms varied distinctly according to the season and the region in which the crops were grown. For example, during a visit to the north-west of Tasmania during the 1936-37 season, the only type of mosaic commonly observed on Bismark was the crinkle without mottle described by Oldaker and Dowson (1929). During the following season, crops of Bismark were examined in the Midlands district, as well as in the north-west. In the Midlands were found symptoms ranging from the very faint indications of mottle caused by virus X on tolerant varieties, to severe leaf-drop-streak, presumably due to virus Y in association with other viruses. There was no evidence of leaf roll. In the north-western region the range of symptoms was more like that recorded during the previous season.

Again, the most vigorous plants of Brownell growing under cool and cloudy conditions exhibited a mild interveinal mottle. This mottle might not be completely masked, even under hot and dry conditions, but it was reduced to negligible intensity. Plants affected by the disease originally diagnosed as "mild mosaic" were often definitely mottled under conditions which masked the interveinal mottle on adjacent "normal" plants. When conditions were favourable for the manifestation of the mild interveinal mottle in "normal" plants, the symptoms on the "mild mosaic" plants were more pronounced. The mottle was brighter or merged into a general yellowing of the leaves, which was accompanied by dwarfing and stiffness, and a rolling of the leaflets suggestive of leaf roll. There were also occasional plants with symptoms of rugose mosaic.

Obviously, the two varieties differed in their reactions, or in their susceptibility to infection by the same viruses. This, and the suggestion that even those plants which appeared healthiest might carry some form of virus infection prompted a thorough study of the reactions of the two varieties to the commoner viruses known to infect potatoes in Australia. These are virus X, which causes the disease known as latent or simple mosaic; virus A which, in combination with virus X, causes crinkle or mild mosaic; virus Y which characteristically produces leaf-drop-streak or rugose mosaic, and the virus of leaf roll. Virus X is transmitted in the field by contact between the foliage of diseased and healthy plants; the other three are transmitted by aphids. All are carried in the tubers to successive vegetative generations of infected plants.

Each of these viruses may, for practical purposes, be considered as a single entity producing characteristic symptoms on any one variety of potato. However, investigations in recent years have shown, particularly for virus X, that different samples of the virus may consistently cause milder or, alternatively, more severe symptoms on susceptible varieties. The available evidence suggests that virus X exists in a number of strains, differing in the severity of the reactions they produce on infected plants (Salaman, 1938). The virus X normally carried by an infected potato plant consists of a mixture of milder and severer strains, and, according to which predominate in the mixture, the symptoms will be relatively mild or severe (Bald and White, 1942). These differences are made manifest by inoculation to such plants as *Datura stramonium,* which are susceptible but less tolerant of infection than most potato varieties.

On many varieties of potato, the milder mixtures of virus X strains do not cause any symptoms, while the more severe produce a mild interveinal mottle. The symptoms are so mild that they were overlooked for a considerable time, and plants containing them were thought to be healthy; so much so that in America virus X, for a period after its presence was discovered, was known as the "healthy potato virus" (Johnson, 1925).

4. Reactions to Virus X.

(i) *Bismark.*

As a virus-free stock of the Silverskin Bismark is now available (Bald, 1944), reactions of this variety to virus X can be clearly defined. The foundation stocks of certified seed have, up to the present time, carried virus X but will shortly be replaced by the X-free stocks. These are becoming known as FX stocks.

The mixtures of virus X strains in Bismark are of average severity. Partial analyses have revealed the presence of mild, medium, severe, and probably necrotic strains, and virus B, which is also a strain of virus X. Inoculation direct from Bismark to *Datura stramonium* generally produces a brighter mottle than is caused by a medium strain alone, but very little necrosis. By selective inoculation, severer strains can readily be concentrated sufficiently to cause necrosis on *Datura*.

The symptoms on Bismark under field conditions are so slight that they are not noticeable except after very careful examination (Plate 1, Fig. 1). They may be totally masked. Often infected plants are a slightly lighter shade of green than virus-free plants, the leaves of the latter having a smoothness of texture and perfection of form not commonly found on plants carrying virus X.

(ii) *Brownell.*

About 2,000 Brownell tubers and plants have been tested for the presence of virus X, and all have been found infected. In addition, many thousands of plants have been examined in the field, and none has been found without the slight mottle produced by virus X, unless the environment has been favourable for the masking of symptoms. The chance of obtaining a Brownell plant free from virus X appears to be very small.

In the strain mixtures of virus X normally carried by Brownell plants, there is a high proportion of severe strains (Bald and White, 1942). Inoculation of the mixtures direct to *Datura stramonium* produces severe mottle and various degrees of necrosis. Occasional Brownell plants carry relatively mild mixtures consisting predominantly of medium strains; but of the mixtures examined, none has been found free from the severe or the necrotic strain.

In Tasmania, the field symptoms produced by virus X are seldom completely masked. They vary from a typical mild interveinal mosaic (Plate 1, Fig. 2) to occasional patches of vein-clearing or a slight break in the even colour of the leaf (Plate 1, Fig. 1) on only a few leaves. At a distance, the shade of colour of the foliage is more yellow than the normal green of virus-free potato foliage.

5. Reactions to Virus A.

(i) *Bismark.*

Bismark is more susceptible to virus A than to the other aphid-transmitted viruses, Y and leaf roll. The disease first described as typical of this variety, "crinkle" (Oldaker and Dowson, 1929), was found to be due to a mixture of viruses X and A. During the 1930's the rate at which crinkle spread was so alarming, it appeared likely that this useful variety might not survive. Apparently healthy crops degenerated within a few seasons under conditions that one might expect to be favourable for the maintenance of healthy stocks.

Officers of the Agricultural Department, examining the progenies of single plant selections, observed slight and fleeting mottles in many families of Bismark thought to be healthy. Analyses at Canberra of their virus-content revealed the presence of virus A and virus X carried separately by different plants. Only under conditions of relatively low light intensity and rapid growth were the symptoms of virus A clearly defined. They were more evident in the greenhouse than in the field.

The symptoms of virus A on Bismark are illustrated in Plate 2, Fig. 1. Centred on the veins against the normal green background of the leaf are lighter coloured areas with nebulous boundaries. There may be slight distortion of the leaf outline. As environmental conditions depart from the optimum for symptom expression, the lighter areas deepen in colour, and the mottle fades or disappears.

The results of the work in the greenhouse at Canberra gave the necessary clue to the composition of the mosaic diseases of Bismark as they existed in the field. In the Midlands, where, as in the greenhouse, conditions were favourable for the development of symptoms, plants were found with symptoms of virus A alone, or of virus A plus X. There was some mottling on leaves of plants carrying the mixed infection, as well as crinkling (Plate 2, Fig. 2). Plants that appeared healthy mostly carried virus X.

The opportunities for the dissemination of crinkle were thus exceptionally favourable. Plants containing virus A could develop crinkle, by contact infection, from plants containing virus X; and plants containing virus X could develop crinkle, by aphid-borne infection, from plants containing virus A. While the single infections passed unnoticed, there seemed no explanation for the exceptional rate of degeneration, unless Bismark were exceedingly susceptible to crinkle; but under conditions favouring the manifestation of symptoms the explanation became obvious.

In the higher country of the north-west, the mottle component of these symptoms was suppressed. Plants containing only virus A appeared almost or quite healthy, and plants with the double infection of X and A exhibited symptoms of crinkle without mottle. Nevertheless, there were similar opportunities for crinkle to develop by the dissemination of viruses X and A.

In the same stock of Bismark, according to the environmental conditions, one disease of the mosaic type, due to X plus A, might be evident; or two diseases if mottling due to A alone were not suppressed; or even three diseases, if the fleeting symptoms due to virus X alone (Plate 1, Fig. 1) were revealed by an exceptionally favourable environment. (As a rule, such an environment has been found only under artificial conditions in the greenhouse.) The presence of virus Y might even further complicate the picture.

(ii) *Brownell.*

This variety is field-immune to infection with virus A, i.e., although the virus may be transmitted to it by artificial methods, plants of Brownell do not as a rule become infected in the field. In previous reports the variety Brownell was said incorrectly to be susceptible to virus A. It was stated that "In the greenhouse, when infected with virus A by grafting, it exhibits a mild mottle, very easily masked by high temperatures" (Bald and Pugsley, 1941). This statement was made because, in earlier experiments, it was found that some weeks after grafting with A-infected scions, growth of a majority of grafted Brownell plants was reduced in comparison with normal controls. The veins on the young leaves were slightly cleared, and mottle on the leaves due to virus X was somewhat intensified or lighter areas might appear in the centres of leaflets along the main veins. These were the only symptoms noticed.

When tubers were obtained from the grafted plants and grown during the following season, they produced plants no different from the controls, and virus A could not be recovered from them. Further extensive series of grafts revealed that in the current season one symptom of infection had been overlooked. An extremely localized necrosis, like that described as apical top necrosis (Scott, 1938) occurred in the stem below the growing tip. Apparently, the same form of necrosis affects the sprouts of tubers to which the virus travels, and kills the growing points. If a sprout of a partially infected tuber is capable of growth it will be free from virus A, and will produce a normal plant. Should infection of a plant ever occur by natural means under field conditions, there could be practically no transmission of virus A through the tubers.

6. Reactions to Virus Y.

(i) *Bismark.*

In the potato-growing areas of Tasmania, virus Y on Bismark is less important than viruses X and A. When it does develop, the symptoms are more severe than those of crinkle due to the mixture of viruses X and A, but it attacks a much smaller proportion of plants. Diseased plants are dwarfed and the leaves are small and rugose. There may be a greater or less degree of mottling according to the environment. Whereas crinkled leaves are relatively flat, and waved at the edges, the rugose leaves of Y-infected plants are characteristically ballooned, with the edges cupped downwards (Plate 2, Fig. 3). Such leaves may be somewhat flattened by the leaflets splitting at the edges, which easily occurs, because the tissues are very brittle. There is little

growth of side shoots on diseased plants, while a few of the lower leaves shrivel prematurely. If the Y-infected plants are free from virus X, there is less shrivelling of the leaf tissues and a milder mottle than if virus X is present. When virus Y is combined with viruses X and A in the same plant, a severe rugose mosaic is caused.

During the season when infection with Y occurs, there is little or no definite necrosis, although inoculated leaves may turn yellow. On such leaves patches of green colour in the form of spots and rings may be left: these are known as "retention patterns." Affected leaves may shrivel prematurely, and mosaic symptoms develop on the younger leaves. The current season symptoms give place in the vegetative descendants of infected plants to a relatively mild rugose mosaic without necrosis.

(ii) *Brownell.*

The reactions of Brownell to virus Y are of the type usually described as leaf-drop-streak and rugose mosaic. As all plants contain virus X, the disease is composite in nature. The current season symptoms include typical necrosis beginning on the underside of the veins, and, if infection occurs fairly early, mottle and rugosity of the upper leaves. Symptoms in subsequent years are dwarfing; rugosity and mottle; yellowing and shrivelling of the lower leaves; and suppression of the growth of the axillary shoots.

In the high country both mottle and necrosis are partly suppressed, and infected plants may consist of one or two erect, but dwarfed shoots with yellow-green rugose leaves (Plate 2, Fig. 3).

As there is no type of mosaic disease intermediate in severity between rugose mosaic and the mild interveinal mosaic due to virus X, there is little chance of mistaking this disease for any other affecting Brownell. Rugose mosaic is not very common in Tasmanian stocks. This does not mean that Brownell (or Bismark) possesses any useful degree of resistance to virus Y, merely that conditions in the more important potato-growing regions of Tasmania are not very favourable for the dissemination of virus Y. Rugose mosaic in Brownell is more frequently found in some districts of Victoria where this variety is occasionally grown.

7. Reactions to Leaf Roll.

(i) *Bismark.*

Bismark is less susceptible to leaf roll than any other commonly grown Australian variety, with the possible exception of Western Australian Delaware (Earliest-of-All). Plants infected with leaf roll have not been seen amongst the better quality crops. When infection does occur, the effects on growth and yield are very severe (Plate 3, Fig. 1). This is an added protection against the dissemination of leaf roll, as few tubers are produced by infected plants to perpetuate the disease in the years following the first full development of symptoms.

Bismark plants infected with leaf roll are severely dwarfed and bushy; the leaves are small, rigid, and yellowish or slightly pigmented. Rolling of the leaves is not the most pronounced symptom.

(ii) *Brownell.*

Leaf roll is the disease principally responsible for the degeneration of Brownell, as Brownell is, of all commonly-grown Australian varieties except Up-to-Date, the most susceptible to infection. The reason why these facts were not recognized when attention was first paid to virus diseases of the potato in Tasmania was that leaf roll in Brownell was thought to be a mosaic disease. When a Brownell plant becomes infected with leaf roll, there is an increase in the intensity of the faint mottle caused by the universal infection with virus X. On the lower leaves some yellowing between the main veins occurs, and the bases of the leaflets near the top of the plant are often yellowish green. Before flowering, the younger leaves are slightly more rugose than corresponding leaves on healthy plants. The mottling and yellowing that accompany leaf roll in Brownell were accepted as the symptoms of greatest diagnostic value, and the disease was given the title "mild mosaic" because of the relative mildness of the mottle, although other symptoms were often severe (Plate 3, Fig. 2).

At maturity Brownell plants infected with leaf roll are dwarfed, but generally not as much as infected Bismark plants. The lower leaves are rolled, but not always strongly so, and the edges may be ruffled. The middle and upper leaves arise from the stem at a more acute angle than is normal, and are slightly mottled or yellow-green in colour. Further, the plants mature earlier than those of normal condition and the tubers, both in size and number, are considerably reduced. Almost inevitably, too, the few small tubers produced are attractive in shape with shallow eyes and bright skin. (A trap for the unwary where seed selection is by tuber examination only.)

8. Diagnosis of Virus Infection.

Control of the virus diseases of potatoes, which is mainly accomplished by seed certification, is an agronomic problem. The methods of certification are little different from those used in the production of pure high quality seed of crops in which the incidence of disease is less important than genetic variation. Both genetic variation and tuber (or seed) transmitted diseases may produce inferior stocks of a crop plant, propagation of which will result in lower yields or poorer quality products. It is the object of certification to eliminate these stocks in favour of those of a higher standard. Preventive methods for the control of virus diseases are necessary, because virus-infected potato plants and their vegetative progeny remain diseased as long as they are grown. There are no curative methods for the elimination of viruses from infected crops.

The first essential in the operation of a potato certification scheme is that farmers and certification officers should be able to recognize virus-infected plants in seed crops. Diagnosis of virus diseases under farming conditions is often more difficult than under experimental conditions in the greenhouse, or in well controlled field trials. Different environments produce variations and changes in intensity of symptom expression, and entirely unrelated causes induce effects that may be mistaken for virus infection in plants free from virus diseases. Some

further discussion may help to make these points clear, and to define more accurately the diagnostic features of the virus diseases characteristic of Bismark and Brownell

Bismark.—The original suggestion (Oldaker and Dowson, 1929) that Bismark is affected in the field by one type of virus disease is at least partly true. Three viruses, X, A and Y, may be involved, but all, singly or in combination, cause diseases of the mosaic type. These diseases, although of the one type, form a graded series from very mild to very severe. The original confusion in diagnosis, and the idea, often propounded, that degeneration is progressive are therefore readily understood. Every combination of the three viruses may occur in the field, and it would be possible to have seven degrees of infection corresponding to the following combinations: X, A, Y, X plus A, A plus Y, X plus Y, and X plus A plus Y. As a Bismark stock degenerates, more and more plants receive the double or triple infections. This would give the impression, if one virus were thought to be responsible, that year by year the disease became progressively more severe.

At the time when work was begun on the regeneration of the Bismark seed stocks, most or all of these combinations of viruses did occur. In the certified seed stocks containing virus X, which have been issued to growers during the last few years, the picture has been somewhat simplified, as plants containing virus X have been rated as healthy, and plants containing virus A alone and the combination A plus Y have not occurred. With the distribution to farmers of FX Bismark seed stocks, which will be possible within the next few years, the problem of milder and severer grades of mosaic disease will again rise. However, the central principle of the present certification scheme, and of the FX scheme (Bald, 1944), according to which mother seed from a central tested stock is supplied each year to certified seed growers, should be adequate to meet the situation. It can be taken for granted that the stocks issued to certified growers will be practically free from virus X, and little infection with virus A or Y is likely. Grown in the Tasmanian Midlands under good conditions, any infection with viruses A and Y will be sufficiently evident for certification officers and growers of certified seed to judge the health of the crops, and rogue diseased plants. It will not be necessary to know which virus or combination of viruses is causing disease as long as the disease is recognizable as a milder or more severe form of mosaic.

Brownell.—The problem of diagnosis in Brownell is somewhat different. It is not easy to confuse the effects of other diseases or adverse conditions with mosaic or crinkle in Bismark, but there are many causes producing a rolling and discolouration of Brownell foliage that might be mistaken for leaf roll. Any injury to the base of the stem or roots of a potato plant may cause rolling of the leaves and some discolouration or pigmentation of the upper leaves. Malnutrition or dry conditions may have similar effects. In spite of this, it is nearly always possible for an experienced observer to distinguish virus leaf roll from rolling due to other causes.

A Brownell plant infected with true leaf roll is more dwarfed than its healthy neighbours, the *lower* leaves are rolled and the foliage is stiff, harsh and rattles when it is shaken. The stems below ground are

generally clean and white, and rather thin, unless some other disease is imposed on the leaf roll. This particular combination of symptoms is not fully reproduced by other causes. Slight infections of *Rhizoctonia* or *Fusarium* Wilt diseases will produce rolling of the leaves, but usually in a manner characteristic of the infection. In such cases the leaves will remain soft in texture and a correct field diagnosis is generally possible. Mechanical injury caused during cultivation, by cutworms, or by high winds partially breaking the stem through at the base, may have a similar effect. If rolling is caused by malnutrition or lack of water, the affected plants will appear in patches, or over a whole field. Further, the full combination of leaf roll symptoms which include dwarfing in comparison with adjacent normal plants accompanied by rolling, and a harsh texture of the lower leaves, will not be present.

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The Relationship between Necrosis and Resistance to Virus Y in the Potato.

2. Some Genetical Aspects.

*By E. M. Hutton, M.Sc.**

Summary.

Fifteen Y hypersensitive phenotypes, isolated as a result of previously published work, are described. These phenotypes and those obtained from selected crosses and selfings can be placed in three definite classes. Of these it is considered that the local necrotic and top necrotic classes are more valuable than the necrotic collapse class, although all three may be of value under field conditions.

Among varieties grown in Australia, Snowflake, Katahdin, and Brown's River appear to be more promising than Bismark, Delaware, Factor, and Sebago as a source of Y hypersensitivity.

Once hypersensitive types have been selected from hybrid progeny, crosses involving them can produce 10-30 per cent. hypersensitive seedlings.

Inheritance of Y hypersensitivity is apparently due to the operation of a recessive allele or alleles, tolerance to the virus being the dominant condition.

1. Introduction.

Hutton and Bald (1945) have shown that it is possible by hybridization to increase the intensity and speed of the necrotic reactions to virus Y found in some potato varieties. It is possible to build up a sensitive reaction like that shown by Epicure to viruses X and A. Data from a few crosses involving these hypersensitive hybrids demonstrated that their production was not fortuitous since the hypersensitive reaction was transmitted to a number of the progeny. Data have now been collected from a series of selected crosses, and these, together with complete data from fifteen originally selected hypersensitive hybrids, give some indications of the possible mode of inheritance of this reaction.

2. Materials and Methods.

The strain of virus Y used throughout these studies was originally obtained from an infected Carman tuber from Victoria. Its reactions on a series of commonly grown potato varieties are similar to those of the usual strain of virus Y found in Australia. It has been kept from year to year in a line of Epicure potatoes, and the symptoms on this host have shown no variation from the usual mild rugose mosaic.

Inoculation of seedlings is done in the glasshouse with a spatula, the plants being first dusted with carborundum, and the inoculum is obtained by grinding infected Epicure leaves with a constant proportion of buffer at pH7. The seedlings are inoculated during late spring and early summer when they are about 15 inches high and growing vigorously.

The determination of the class to which the reaction of any particular seedling belongs is made one month after inoculation, when flowering is usually completed and tuberization has commenced. The average reactions of the original series of seedlings, selected for hypersensitivity to virus Y from selfed Katahdin and the crosses Brown's River x Katahdin and Snowflake x Katahdin, have been determined from replicated tests of each inbred or hybrid at six different times over

* An officer of the Division of Plant Industry.

two and a half years. The three promising types of necrotic reactions to virus Y present in this group of hybrids, as exemplified by 1-48, 1-106, and 2-404. have already been described in detail by Hutton and Bald (1945).

To obtain a preliminary idea of the type of inheritance of the hypersensitive reaction to virus Y, thirteen crosses and four self pollinations were made involving certain of the original hybrids and common potato varieties. Altogether, 1,750 vigorous tuber progeny from the resultant seedlings were raised and tested in the glasshouse during the spring and early summer of 1944. Based on the previous experience with the original series, the phenotypic reactions to virus Y of this second series were grouped into six classes, viz., mottle, systemic necrosis, leaf drop streak, necrotic collapse, top necrosis, and local necrosis. It is assumed that this series represents an increasing degree of sensitivity to virus Y. These classes could have been subdivided further into mild and intense subdivisions, but this was found an unnecessary complication since the reactions of several hundred duplicates have shown that differing environmental conditions can affect the intensity of reaction. With few exceptions it was possible to place the reaction of a hybrid or its tuber progeny in a definite class.

3. Hypersensitive Reactions of the Selected Hybrids from the Original Crosses.

As a result of the preliminary work on the development of hypersensitivity to virus Y by suitable hybridization described by Hutton and Bald (1945), fifteen promising potato hybrids were eventually selected. In Table 1 is given a summary of these hybrids, their breeding and reactions to virus Y, and the extent of protection against this virus as shown by the reactions of the uninoculated tuber progeny from inoculated plants.

TABLE 1.—SUMMARIZING THE REACTIONS TO VIRUS Y OF FIFTEEN SELECTED HYPERSENSITIVE POTATO HYBRIDS.

Order of Sensitivity.	Selection Number.	Breeding.	Average Reaction to Leaf Inoculation.	Average of Reactions in Tuber Progeny of Inoculated Plants.		
				Non-emergence.	Emerged Healthy.	Emerged Diseased.
				%	%	%
1	1–15	BR* x K†	Localized ..	..	100	..
2	2–404	SF‡ x K	Mainly localized	30	70	..
3	2–411	SF x K	,, ,,	23	77	..
4	3–445	Selfed K	Localized ..	30	70	..
1	2–353	SF x K	Top necrotic	..	100	..
2	1–106	BR x K	,, ,,	..	100	..
3	2–355	SF x K	,, ,,	..	100	..
4	2–349	SF x K	,, ,,	..	100	..
5	1–129	BR x K	,, ,,	6	94	..
6	1–176	BR x K	,, ,,	13	26	61
1	2–240	SF x K	Necrotic collapse	..	100	..
2	1–48	BR x K	,, ,,	7	60	33
3	2–241	SF x K	,, ,,	..	50	50
4	1–221	BR x K	,, ,,	8	20	72
5	1–142	BR x K	,, ,,	..	50	50

* BR = Brown's River. † K = Katahdin. ‡ SF = Snowflake.

The hybrids are grouped in this table into three classes of reaction to virus Y, viz., localized, top necrotic, and necrotic collapse. In each class the hybrids are placed in order of decreasing sensitivity. It is only under quick-growing spring and early summer conditions that a true picture of the reaction to leaf inoculation can be obtained. Under slower growth conditions, as in autumn, the tendency is for most of the hybrids described in Table 1 to give a localized necrotic reaction; 1-15 and 3-445 are the only two which have consistently given localized reactions in all tests so far conducted. It has been found that only hybrids possessing one or other of the three classes of necrotic reaction described are protected against this virus. From Table 1 it can be seen that the degree of protection against virus Y introduced into the plant by leaf inoculation is largely dependent on the type of necrotic reaction. From this evidence it appears that the necrotic collapse type is the least effective, although hybrids 2-240 and 1-48 are considered valuable since the tuber progeny of infected plants of the former are healthy, while those of 1-48 which emerge diseased die off rapidly in seven to ten days without making more than a few inches of growth. The other three hybrids of this class not only produce a relatively high percentage of diseased tuber progeny, but the plants from such progeny often produce large, leafy plants which take five to six weeks from emergence to die. During this period they might provide a reservoir of virus Y for dispersion to healthy plants by aphids. The same applies to the only unsatisfactory hybrid, 1-176, in the top necrotic class. However, even these less satisfactory types may be quite valuable under field conditions, since a few limited experiments have shown that inoculations by the aphid vector often causes a more rapid reaction than with leaf inoculation, and that any tubers produced are generally too small for seed.

Table 1 shows that in these hypersensitive hybrids there is a gradation in Y sensitivity. Which are the most valuable under field conditions has yet to be decided. It appears that the local necrotic and top necrotic classes will be the most valuable, although it is possible that all three classes will provide an effective barrier to virus Y under field conditions.

4. Inheritance of the Hypersensitive Reaction in a Selected Series of Crosses and Inbreds.

In Table 2, difference of genotype amongst four pollen fertile types used in the series of crosses is shown by selfing, sib-mating between two of them, and outcrossing one of them. As stated previously, 2-404 and 2-411 have mainly a localized reaction to virus Y, whereas Bismark reacts with intense mottle and some rugosity. The hybrid 2-362 is a Snowflake x Katahdin cross and reacts with typical leaf drop streak and mottle to virus Y.

The contrast between 2-404 and 2-411 on the one hand and 2-362 and Bismark on the other with respect to proportions of non-sensitive and hypersensitive phenotypes to virus Y obtained by selfing is striking. As shown in Table 2, a third of the progeny from selfing 2-411 is hypersensitive compared with a fifth from selfing 2-404. Selfing Bismark and 2-362 resulted in no hypersensitive phenotypes. Sib-mating of 2-404 and 2-411 resulted in the production of 27·5 per cent.

of hypersensitive types, a figure approximately half-way between those obtained by selfing these two hybrids. Outcrossing 2-404 to Bismark gave no significant reduction in the percentage of hypersensitive phenotypes expected from 2-404 selfed, but there is a distinct reduction to almost one-third when 2-404 is sib-mated to 2-362.

TABLE 2.—PROPORTIONS OF Y TOLERANT AND Y SENSITIVE PHENOTYPES OBTAINED FROM SELFING AND INTERCROSSING FOUR MALE PARENTS.

Breeding.	Tolerant Phenotypes.			Hypersensitive Phenotypes.			Total Number Tested.	Number giving Hypersensitive Reaction.	Hypersensitive.	Ratio of Hypersensitive to Tolerant.
	Mottle.	Systemic Necrosis.	Leaf Drop Streak.	Necrotic Collapse.	Top Necrosis.	Local Necrosis.			%	
2–404 selfed ..	37	5	4	8	1	2	57	11	19·3	1 : 4
2–411 selfed ..	18	14	6	6	6	8	58	20	34·5	1 : 2
2–362 selfed ..	37	nil	5	nil	nil	nil	42	nil	nil	..
Bismark selfed ..	94	..	..	..	..	..	94	..	..	..
2–404 x 2–411 ..	12	35	3	nil	8	11	69	19	27·5	1 : 3
Bismark x 2–404 ..	43	47	36	24	5	nil	155	29	18·7	1 : 4
2–362 x 2–404 ..	116	12	27	8	4	nil	167	12	7·2	1 : 13

TABLE 3.—PROPORTIONS OF Y TOLERANT AND Y SENSITIVE PHENOTYPES OBTAINED FROM EIGHT CROSSES INVOLVING SIX DIFFERENT FEMALE PARENTS AND TWO MALE PARENTS.

Breeding.	Tolerant Phenotypes.			Hypersensitive Phenotypes.			Total Number Tested.	Number giving Hypersensitive Reaction.	Hypersensitive.	Ratio of Hypersensitive to Tolerant.
	Mottle.	Systemic Necrosis.	Leaf Drop Streak.	Necrotic Collapse.	Top Necrosis.	Local Necrosis.			%	
41956 x 2–404 ..	112	27	21	39	11	5	215	55	25·6	1 : 3
Epicure x 2–404 ..	76	34	33	28	11	5	187	44	23·5	1 : 3
1–142 x 2–404 ..	56	6	10	2	9	1	84	12	14·3	1 : 6
Snowflake x 2–404 ..	83	32	23	5	1	4	148	10	6·8	1 : 13
Snowflake x Bismark	14	4	11	1	2	1	33	4	12·1	1 : 7
1–142 x Bismark ..	63	7	35	nil	12	1	118	13	11·0	1 : 8
Delaware x Bismark	121	nil	18	5	nil	nil	144	5	3·5	1 : 28
Factor x Bismark ..	71	2	1	1	nil	nil	75	1	1·3	1 : 74

In Table 3 the results obtained from crossing six different female parents with two male parents are given. The reactions to virus Y of the male parents 2-404 and Bismark have already been described. The female parent Epicure is tolerant to virus Y, 1-142 reacts with necrotic collapse, Snowflake reacts with a slow but fairly severe necrosis, 41956 gives a systemic necrotic reaction to Y, and Delaware and Factor usually react with a distinct mottle together with some necrosis.

The proportions of tolerant and hypersensitive phenotypes to virus Y obtained from the eight crosses are shown in Table 3. In Table 2, the proportions of these phenotypes resulting from selfing the two male parents 2-404 and Bismark are given. The percentages of hypersensitive phenotypes in the crosses are greater, with the exception of those involving 1-142 and Snowflake with 2-404, than the percentages obtained on selfing the corresponding male parent. These results give an indication of the possible genotypes with respect to Y hypersensitivity of the six female parents.

The two crosses with the female parent 1-142 are interesting in that most of the hypersensitive progeny are in the top necrotic class. That this may be a characteristic of this female parent is borne out by the results of a cross between it and 2-411. Of the 92 seedlings produced, 6·5 per cent. were hypersensitive, all of them being in the top necrotic class.

Considering the results in Table 3 as a whole, the four crosses using 2-404 as the male parent produced 19 per cent. of phenotypes hypersensitive, whereas the four using Bismark as the male parent yielded 6 per cent. When consideration is given in these crosses to the percentages of the two most valuable classes, viz., top necrotic and local necrotic, 2-404 produced 7·4 per cent. of these types and Bismark 4·3 per cent., a difference which is not as great as when the necrotic collapse class is also included. As previously explained, the ultimate value of the three types of hypersensitive phenotypes will only be determined by infection under field conditions over several seasons in different localities. It is assumed, on the basis of the results in Table 1, that field tests will eliminate a large percentage of the necrotic collapse class, but will prove the value of the top necrotic and local necrotic classes.

5. Discussion.

The results so far obtained have shown that certain genotypes conditioning hypersensitivity to mechanical inoculation with virus Y have been isolated in hybrids involving common potato varieties. More work is needed to determine the actual constitution of these genotypes. Among varieties grown in Australia, it appears that Snowflake, Katahdin, and Brown's River have the most promising genotypes for the production of Y sensitive phenotypes, whereas Bismark, Delaware, and Factor are less promising in this respect. That the variety Sebago also has a poor genotype from this point of view is evidenced by the fact that only one Y hypersensitive seedling was found in 108 seedlings raised from the cross Sebago x 1-50, the latter parent giving leaf drop streak and mottle to Y inoculation.

It appears that most potato crosses will give a percentage of the Y hypersensitive phenotypes described, the percentage obtained depending on the genotypes of the parents. However, once such valuable types are isolated, even though they may come from a cross poor in this respect, further crosses involving the selected hypersensitive hybrids would be expected to yield a fair percentage of the types required. Whether it will be possible by selected hybridizations to raise the percentages of such Y hypersensitive progeny in crosses above the best of those quoted in Tables 2 and 3 remains to be seen.

Modern work on inheritance in the potato tends to show it to be an autotetraploid. Cadman (1942) found that inheritance of the necrotic reaction to virus X was tetrasomic, and that it was associated with the dominant allele of a gene, most necrotic reacting varieties being simplex. From the work of Stevenson, Schultz, and Clark (1939) inheritance of immunity to virus X appears to be tetrasomic and due to two dominant complementary genes. Whether the inheritance of hypersensitivity to virus Y can be fitted into the general picture of autotetraploid inheritance as shown by the two papers mentioned depends largely on whether a more clear-cut determination of hypersensitivity can be made in the seedlings of various crosses. The whole position is complicated to some extent by the fact that, previous to the work of Hutton and Bald (1945), no hypersensitive types, as described, were known, as far as could be ascertained from available literature. From the evidence so far presented, it appears that the character is dependent on a recessive allele or alleles, and that the dominant condition is one of tolerance to virus Y. It is possible that the nulliplex condition is represented by both the localized and top necrotic reactions, and the simplex by the necrotic collapse types. This assumes a cumulative genic effect from the quadriplex condition of tolerance through to the nulliplex condition of hypersensitivity.

In Tables 2 and 3 the bulked ratios of hypersensitive phenotypes to non-sensitive phenotypes vary from 1:2 to 1:74, with a tendency for distinct groups of ratios, viz., 1:3, 1:7, 1:13, 1:28, and 1:74. The narrower ratios come from consideration of the progeny of selfed Y hypersensitive hybrids, intercrosses between such hybrids, and outcrosses with varieties apparently possessing factors for Y hypersensitivity in a recessive condition. There appears to be no reason why such results will not fit auto-tetraploid inheritance of a single recessive allele when not only more data are available, but when more is known of the actual reaction, particularly its relation to field immunity.

6. Acknowledgments.

The writer gratefully acknowledges the help given by Dr. J. G. Bald of the Division of Plant Industry, Canberra, and Dr. I. Watson of the University of Sydney, in critically examining the manuscript of this article.

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The Effect of Flax Straw Maturity on the Microscopic Structure and Dimensions of the Ultimate Fibres.

*By Winifred M. P. Cook, B.Sc.**

Summary.

Measurement of the dimensions of ultimate flax fibres showed that, when the straw was immature and the plants still flowering, the fibres were small but very irregular. When the seed bolls were formed and the stems beginning to turn yellow, the fibres were much more regular in shape but were still small and well filled (i.e., the lumen was small). At this stage the spinning quality was at its highest. As the straw became more mature, the fibres increased to their maximum size with a corresponding decrease in quality.

When samples were taken from three positions in the stem, the smallest and most irregularly shaped fibres were found in the head, and there was a gradual increase in size down to the butt.

1. Introduction.

Fibre evaluation tests on a series of fourteen flax harvests (taken at weekly intervals) from the Waite Agricultural Research Institute, S.A., in 1942, showed that the quality of the fibre obtained, was considerably affected by straw maturity. Details of this straw at different stages of maturity, together with the corresponding line fibre yield and quality, are shown in Table 1. Samples from the 5th to the 8th harvests gave fibre of the best potential spinning quality but, as the ripeness of the straw increased further, the fibre became dry and harsh and the spinning quality decreased rapidly. In view of these changes which take place with increasing maturity, it was considered that valuable information on fibre properties might be obtained by investigating microscopically the changes which occurred in the size and shape of transverse sections of the ultimate fibres in the above straw. This work was therefore undertaken and in addition a study has been made of the effect of position in the stem on fibre dimensions.

Searle (1923) found that during the ripening of normal flax crops there was only a very slight increase in fibre dimensions, but, in more thinly sown stands, both the number and area of the ultimate fibres increased up to the end of the vegetative period. In this Australian trial, the seeding rate (68 lb. per acre) was much less than that normally used in England (110 lb. per acre).

For a long time, the spinning quality of flax has been considered to be related to the microscopic characteristics of the ultimate fibres. It has been stated (Hardy and Wolf, 1939) that the spinning quality of textile fibres is dependent, to a large extent, on the cross sectional shape. For wool and possibly flax, small round fibres are superior to those that are large and irregular. In addition, graders consistently

*An officer of the Flax Section, Division of Forest Products. This section has now been merged with the Flax Research Laboratory, which the Council is establishing in co-operation with the Flax Production Committee of the Department of Supply and Shipping.

state that one of the most important characteristics of quality is the fineness to which flax can be hackled. This depends, ultimately, on the size of the individual fibres.

Microscopic examination is particularly important in attempting to correlate fibre dimensions with straw characteristics and fibre quality, because the ultimate fibres can be measured directly and a quick and accurate assessment of their size and shape can be made. Furthermore, this information can be obtained from small samples, the normal grading of which would be impracticable.

TABLE 1.—THE APPEARANCE OF THE STRAW AT VARIOUS STAGES OF MATURITY ALSO LINE YIELD AND QUALITY OF THE RESULTANT FIBRE.

Date of Harvest.	Number of Harvest.	Stage of Growth.	Line Yield of Fibre.	Grade of Fibre and Grader's Remarks.
			%	
2.10.41	3	About the middle of the flowering period. Some bolls fully formed	18·6	C.—Shivy, immature. Would improve to D on hand scutching
16.10.41	5	Flowering complete. About ½ plant yellow	20·6	F.—Good flax. Would improve to K on hand scutching
30.10.41	7	Seed bolls commencing to change colour	23·3	G.—Good type of thread flax but slightly harsh. Would improve to J
6.11.41	8	Stems light-straw colour—practically all leaves fallen. Seed bolls beginning to rattle	21·7	F.—Thread type but quality is decreasing. Further hand scutching would only improve fibre about one grade
13.11.41	9	All leaves fallen from stem. Seeds becoming firm	22·2	F.—Harsh but very strong. Good type of thread flax
20.11.41	10	.. ..	21·2	E.—Thread flax. Would improve one grade only
27.11.41	11	Seed fully matured. Straw golden shade. Some bolls fallen	22·8	E.—Thread type but harsher and losing spinning quality. No improvement with hand scutching
4.12.41	12	Plants dry and rather brittle and harsh. Cells of plant probably dead	22·6	E.—Harsher than 11 and less spinning quality
18.12.41	14	Many bolls fallen. Considerable quantity of seed lost during pulling	21·7	D.—Very harsh thread flax. No improvement on hand scutching

2. Experimental.

Fibre samples from harvests 3, 5, 7, 8, 9, 10, 11, 12, and 14 were used. Sections could not be obtained from fibre in harvests 1 and 2 because the straw was so immature that it did not ret properly and had little commercial value. Sections were cut 12 inches from the butt in all samples, and in the 3rd and 14th harvests, sections were cut from the head and butt also to investigate the variation in fibre size along the stem.

(i) *Sampling.*—The specimens for examination were chosen by random selection from the bulk sample. Five small stricks were selected from each sample. Lengths of 4 inches were obtained from each strick by cutting them 10 inches from the butt and using the next 4 inches. The sections were cut in the centre of this so that they were always 12 inches from the butt. When fibres from the head and butt were also required, they were cut as near these positions as possible.

(ii) *Section Cutting and Staining.*—The transverse sections were cut on a new fibre microtome (see Appendix). This is a modification of Hardy's (1935) device and has been designed and made at this Division. The method was similar to that used by Hardy, the sections when cut being embedded in a thin film of celluloid. They were stained for ten minues in Congo Red (1 per cent. aqueous), washed in water and mounted in dilute glycerol. They were then placed in a projection microscope and the contours of 20 fibres were drawn from each section, using a magnification of 490.

(iii) *Measurement of Fibres.*—After the ultimate fibres had been traced, measurements of both major and minor axes were made by means of a millimetre scale.

3. Results.

All results were statistically analysed as to the effect on fibre dimensions of (i) time of harvest and (ii) position in the stem. The results are shown in Tables 2 and 3.

4. Examination of Results.

(i) *Time of Harvest.*

Major Axis Measurements.—The effect of time of harvest on these measurements was highly significant. There was no difference from the 3rd to the 7th harvest ($22 \cdot 5$—$21 \cdot 8\mu$) but from the 7th to the 12th ($21 \cdot 8$—$25 \cdot 9\mu$) there was a very marked increase in the major axis. From the 12th to the 14th harvest the measurements did not alter appreciably.

Minor Axis Measurements.—These results were similar to those for the major axis, the greatest increase being between the 8th and 12th harvests ($17 \cdot 0$—$19 \cdot 3\mu$).

When both major and minor axes were considered, there was no noticeable difference in the size of the fibres from the 3rd to the 7th harvest. During these early harvests it is possible that the plant was growing rapidly and, apart from the fibres filling out, there was little opportunity for them to increase in size. The most rapid increase took place from the 7th harvest, the fibres reaching their maximum size about the 12th harvest (Table 2). At the latter stage, growth of the plant had ceased. This increase in fibre size may be partly due to the increase in hemicellulose content during the ripening of the straw, which has been shown to occur in fibre from this trial (Couchman, 1944).

TABLE 2.—SHOWING THE DIMENSIONS OF ULTIMATE FIBRES AT EACH TIME OF HARVEST.*

Harvest Number.	Major Axis.	Minor Axis.
	μ	μ
3	22·5	15·5
5	22·0	15·7
7	21·8	16·4
8	23·7	17·0
9	24·3	18·3
10	25·1	19·0
11	24·7	18·8
12	25·9	19·3
14	26·2	18·1

* Each measurement is the mean of 100 fibres.

Plate 4, Fig. 1, shows the fibres from the 3rd harvest. They are small and very irregular in shape. Fibre from the 7th harvest can be seen in Plate 4, Fig. 2. At this stage the spinning quality is high and the ultimate fibres are small, regular in shape and have small lumens. Plate 5, Fig. 1, is from the 12th harvest and shows the mature fibres to be large, hexagonal in shape, and containing a small lumen.

(ii) *Variation in Size of Ultimate Fibres in the Stem.*

For both the 3rd and the 14th harvests there was a highly significant difference in fibre size between the three positions in the stem (see Table 3).

TABLE 3.—DIMENSIONS OF FIBRES IN THREE POSITIONS OF STEM IN 3RD AND 14TH HARVESTS.*

Harvest Number.	Axis.	Stem Position.		
		Head.	Middle.	Butt.
		μ	μ	μ
3	Major	20·4	22·5	28·5
	Minor	10·4	15·5	15·8
14	Major	21·3	26·2	31·0
	Minor	14·5	18·1	17·8
Mean	Major	20·8	24·3	29·8
	Minor	12·5	16·8	16·8

* For both harvests, each measurement is the mean of 100 fibres.

For major axis, the mean values for the head ($20 \cdot 8\mu$) gave the smallest measurements, then middle ($24 \cdot 3\mu$) and finally, butt ($29 \cdot 8\mu$). These differences were all highly significant. The head measurement for the minor axis was again significantly lower (at the 1 per cent. level) than the other two positions. There was, however, no difference between the middle and the butt.

5. Conclusions.

(i) When the individual fibres (in cross section) were small and rounded in shape, i.e., 5th to the 7th harvests, fibre of the best spinning quality was produced. With increasing maturity of the straw, fibres became larger and there was a serious deterioration in quality. When Searle's (1923) results are considered with these, it seems possible that the effects of straw maturity might be partly overcome by the use of heavier seeding rates.

(ii) The smallest and most irregular fibres were found in the head and there was apparently a progressive increase in size to the butt.

6. Acknowledgments.

Grateful acknowledgment is made to Miss N. Ditchburn for analysing the results statistically, and to Miss J. F. Couchman for her help throughout the experiment.

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Appendix.

Fibre Microtome.

In order to study the microscopic structure of flax effectively, it is necessary to be able to cut good transverse sections of the fibre. Such sections are very difficult to obtain using the standard types of microtome, which involve either paraffin or celloidin embedding. Both of these methods are unsuitable because it is very difficult to keep the fibres in position in the embedding medium, and 2-3 days are required for the complete process.

For these reasons a special fibre microtome (Plate 6) has been designed in this laboratory.* Although planned primarily for flax, it would be useful for cutting sections of any textile fibre.

Hardy (1935) invented a simple hand microtome for fibres. This was used fairly successfully at this laboratory for some time. The new fibre microtome, however, while incorporating the same general principle as adopted by Hardy, has been designed to overcome some of the disadvantages of the original device and to increase its accuracy.

Briefly, the principle of Hardy's instrument was as follows. A tuft of fibres was inserted into a slit in a metal plate. A plunger operated by a propeller screw forced the fibre through the slit, the thickness of the section being controlled by the amount the screw was turned. A viscous celluloid solution was applied over the surface of the fibres and plate. When the celluloid dried, a sharp razor blade held in the hand was used to cut a transverse section of celluloid with the fibres embedded.

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* Details of the microtome were designed by Mr. L. Santer, to whom grateful acknowledgment is made.

In the new microtome, the movement of the razor blade has been made mechanical in order to overcome the irregularities and difficulties experienced when the blade was held in the hand.

This has been brought about by mounting the blade in a holder which moves in a V shaped groove. By means of a series of screws, the position of the blade can be altered so that its most effective cutting edge is always parallel to the table (Plate 5, Fig. 2).

A micrometer screw has replaced the propelling screw of the older apparatus. An arm is attached to the ratchet of the micrometer, the movement of this arm being restricted by an adjustable rider. The rider is set so that one complete movement of the arm advances the plunger 5 μ. In this way, sections of 5 μ thickness or multiples of 5 can be readily obtained. This instrument is also constructed so that the microtome parts are mounted on a firm metal base which gives the machine rigidity and also makes it easy to handle.

A Note on Vegetative Propagation and Tree Form in *Duboisia* Spp.

*By K. Loftus Hills, M.Agr.Sc.**

Summary.

Various methods for the vegetative propagation of *Duboisia* spp. have been tried. Reproduction from stratified root cuttings was partly successful, and from softwood tip cuttings, very successful, provided certain conditions of temperature, humidity, and rooting medium were observed.

Propagation of contrasting young tree types of *D. Leichhardtii* demonstrated that such type differences were sustained in the vegetative progeny.

1. Introduction.

The vegetative propagation of material is an essential prerequisite for an experimental programme designed to discover the optimum conditions for the cultivation of the alkaloid-bearing species *Duboisia myoporoides* and *D. Leichhardtii*. Many Australian native species are notoriously difficult to propagate asexually, and apparently *Duboisia* is no exception. An outline is given below of the efforts made to propagate the species, and an illustration is given of the application of a successful method to the propagation of contrasted young tree forms of *D. Leichhardtii.*

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2. Methods of Propagation.

Both species are often naturally reproduced by means of root suckers, and in several cases groves of young *Duboisia* suckers have been observed still attached to the root systems of parent trees. However, when such young growths were lifted with a section of parent root attached they failed to establish satisfactorily. It is assumed that the shoots, in the early stages at least, are dependent upon the parent root system, and do not readily form adventitious roots.

Attempts have been made both at Nambour, Queensland, and Canberra, A.C.T., to propagate young and old trees from hardwood cuttings. Various rooting media were used, the cuttings were set under humid conditions and in the open, with and without hormone treatment, at different times of the year, and others were stratified for several months before setting. From all of the above treatments only an occasional cutting rooted.

Both unstratified and stratified root cuttings were set. The former failed to root but the latter were more promising. Of 40 *D. Leichhardtii* cuttings, 20 produced both shoots and roots, and 9 produced shoots only. Of 50 *D. myoporoides* root cuttings, 18 yielded both shoots and roots, and 17 shoots only.

The procedure called " stooling " was also tried. Young plants were cut back to ground level and as the young shoots emerged they were hilled up with soil. After several months, there was no sign of the formation of adventitious roots.

Softwood cuttings were set repeatedly under various conditions until a suitable combination of medium, plant part, and atmospheric conditions was discovered. Although the limits within which these factors may be varied successfully have not been defined precisely, it has been demonstrated that if certain conditions are met successful propagation is assured.

The medium used was a coarse river sand with a mean particle size of about one millimetre. It was washed with tap water before use to remove any surplus organic matter. Green tip cuttings about 4 inches in length were taken from either main or side branches of one to two year old trees. They were then trimmed, leaving the growing points and two pairs of terminal leaves intact. The cuttings were placed overnight in a solution of a commercial root-promoting substance, (" Hortomone A ") and, after a thorough rinsing with tap water, were set to a depth of 3 inches around the periphery of 7-in. earthenware pots filled with moist said. The pots were then placed in a glass chamber under conditions of high humidity which was maintained by flooding the floor of the chamber with water. After three months at 60°F.-70°F. the majority of the cuttings had rooted.

The figures given in Table 1 are typical of those obtained under glasshouse conditions at Canberra. Comparable results were obtained at Nambour, Queensland, during the cooler months, but during the summer fungal attack resulted in almost 100 per cent. mortality of

cuttings set in pots under bell jars. Most of the cuttings used were from trees less than two years old. However, further evidence suggests that similar cuttings from fully mature trees will root equally well. Somewhat lower humidities have been used with success, particularly at the lower temperatures.

TABLE 1.—THE ROOTING OF SOFTWOOD CUTTINGS OF *Duboisia* SPP.

(*a*) *D. Leichhardtii* set in September, and examined three months later—

Treatment Prior to Setting.	Rooted.	Calloused.	Dead.
	%	%	%
Immersion in tap water for 18 hours	40	4	56
Immersion in 0.5 per cent. aqueous solution of "Hortomone A" for 18 hours	53	7	40
Immersion in 1 per cent. aqueous solution of "Hortomone A" for 18 hours	70	0	30

(*b*) *D. Leichhardtii* set in February, and examined six weeks later—

Treatment Prior to Setting.	Rooted.	Calloused.	Dead.
	%	%	%
Immersion in tap water for 18 hours	17	57	17
Immersion in 1 per cent. aqueous solution of "Hortomone A" for 18 hours	33	63	3
Immersion in 2 per cent. aqueous solution of "Hortomone A" for 18 hours	63	33	3

(*c*) *D. myoporoides* set in February, and examined six weeks later—

Treatment Prior to Setting.	Rooted.	Calloused.	Dead.
	%	%	%
Immersion in tap water for 18 hours	30	63	7
Immersion in 1 per cent. aqueous solution of "Hortomone A" for 18 hours	70	23	3
Immersion in 2 per cent. aqueous solution of "Hortomone A" for 18 hours	73	17	3

Callus formation and the subsequent emergence of roots was more rapid in cuttings treated with root-promoting substances. The roots were also larger and more numerous on such cuttings. Plate 7 shows this clearly. On the left are untreated cuttings with markedly smaller roots than those on the right, which have been treated with successively

higher concentrations of "Hortomone A." A secondary effect of the quicker callus and root formation is a lowering of the death rate owing to a lessening of the time during which fungi are likely to attack the cuttings. In most cases callus formation was ultimately followed by root development.

3. Contrasting Young Tree Forms of *D. Leichhardtii*.

Considerable variation in tree form was observed in a twelve months' old plantation of *D. Leichhardtii* established at Canberra. Some of the young trees were sparsely leaved and the branches were not numerous, whilst others were very leafy and had many branches. All stages between the two extremes were apparent. The contrasting types are pictured in Plate 8, Fig. 1.

It was possible that the differences observed were the result of varying soil fertility.* To determine if this were so, cuttings were struck from the trees in Plate 8, Fig. 1, by the method described. Twenty of the rooted cuttings were subsequently transplanted into the field, where the two types were arranged alternately. After six months the parental differences were obvious in the vegetative progeny, as may be seen in Plate 8, Fig. 2, which shows two typical contrasting pairs.

The practical importance of such differences in plant form is illustrated by the fact that at Canberra fifteen-month-old trees of the leafy type yielded over twice the weight of dry leaf per acre that did sparse trees of the same age.

4. Conclusions.

(i) *Duboisia myoporoides* and *D. Leichhardtii* may be propagated vegetatively by means of softwood tip cuttings.

(ii) Differences in form between young trees of *D. Leichhardtii* observed at Canberra are maintained in their vegetative propagants.

5. Acknowledgments.

Thanks are due to Dr. C. Barnard for suggestions concerning methods, and to Miss M. E. Williams, whose persistence was largely responsible for the solution of the propagating problem.

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* It is not considered likely that disease was responsible for the differences observed, for both trees were quite healthy and normal in appearance. In any case it is doubtful if any known disease could simulate such differences in plant form.

A Preliminary Report upon Variation in the Nature and Quantity of the Main Alkaloids in *Duboisia myoporoides* and *Duboisia Leichhardtii*.

By K. Loftus Hills, M.Agr.Sc., E. M. Trautner, M.D., B.Sc.† and C. N. Rodwell, B.Sc.†*

The investigation reported in this paper is a joint project of the Division of Plant Industry and the Department of Physiology, University of Melbourne. The chemical work was financed by grants from the Commonwealth Research Fund and the National Health and Medical Research Council.—Ed.

Summary.

The extent and nature of the variation in the alkaloids of *Duboisia myoporoides* and of *Duboisia Leichhardtii* have been investigated by examining 82 samples of leaf collected from individual trees scattered throughout the known distribution areas of the species. Certain of the trees were sampled on different occasions, and young plantation material growing at Nambour (Q'ld) and Canberra (A.C.T.) was sampled at regular intervals over a period of a year or more.

Considerable variation in both the quantity and nature of the alkaloids was revealed. It is evident that the frequency distribution of alkaloid population types controls the alkaloid content of aggregate samples from natural stands, and that, although considerable variation may occur from time to time in individual trees, the balance, on the average, favours hyoscine in the northern section of *D. myoporoides*, and hyoscyamine in *D. Leichhardtii* and the southern area of *D. myoporoides*.

Changes occurred in plantation material during the twelve months, the major alkaloid changing from hyoscine to hyoscyamine and vice versa. Nevertheless the average composition of both species throughout the year was much the same as that of natural stands.

The assay method used, which has several novel features, is described, and its interpretation discussed in some detail.

1. Introduction.

Considerable controversy took place during the latter part of the nineteenth century, concerning the nature of the alkaloids present in the leaves of *Duboisia myoporoides*. Some workers named hyoscine as the principal or only alkaloid, and others, hyoscyamine. In reviewing these divergent results Petrie (8) suggested that the explanation might be in the fact that the plant is capable of producing both alkaloids, and that variations in conditions of growth might determine the balance between them. Subsequently, Martin (1) examined samples from a number of Australian localities and reported that hyoscine was always present, but that hyoscyamine was never found. Later, Barnard and Finnemore (3), after examining bulk samples from a number of districts, concluded that *Duboisia myoporoides* growing north of Gosford (N.S.W.) contained hyoscine, and that the major alkaloid south of Gosford was hyoscyamine.

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Petrie (8) found that hyoscyamine was the principal alkaloid in *D. Leichhardtii,* although hyoscine, nor-hyoscyamine, and atropine were also present. Barnard and Finnemore (3) reported that hyoscyamine was the preponderating alkaloid in the several bulk samples which they examined, but that hyoscine was also present in some samples. Variation has also been reported in the percentage of total alkaloids in each species, but lack of uniformity in assay methods lessens the value of published figures.

The samples examined by previous workers comprised leaf from an unknown number of trees. In order to determine precisely the extent and nature of the variation in the alkaloids, an individual tree survey covering the known distribution areas of both species was undertaken. Other factors which might affect the quantity or nature of the alkaloids were kept as constant as was practicable. The number of trees examined was limited by the assay facilities available, and is inadequate for a quantitative evaluation of the variation disclosed. An equally important object of the survey was to provide material for selection and propagation.

Experiments were also carried out to determine whether the alkaloids of a particular tree are constant in nature and amount, and whether they are a function of the inherited nature of the tree, of its environment, or of both. Individual trees in natural stands were sampled at different times, and plantation material grown from similar seed, but in contrasting environments, was examined at regular intervals.

2. Collection and Handling.

Leaf samples were collected, during the flowering-fruiting season, from individual trees scattered throughout the known distribution areas of *D. myoporoides* and *D. Leichhardtii.* Certain of the trees were sampled again a year or more later, whilst young plantation plots at Nambour (Q'ld.) and Canberra (A.C.T.) were sampled at regular intervals over a period of one to two years. The samples from natural stands were dried in the shade, or in hessian bags exposed to the sun, and the plantation samples in artificial driers at temperatures of 106° to 116°F. Although drying conditions were not uniform, they are not considered likely to be an important cause of variation, as unpublished data suggest that variation in drying conditions within the atmospheric range of temperatures and humidities does not appreciably affect the nature of the alkaloids, and only slightly affects the quantity. After drying, the samples were ground and stored in airtight tins to await assay. The effect of storage upon the nature and quantity of the alkaloids is the subject of a separate investigation, but analogy with the behaviour of other alkaloids suggests that marked changes are not likely to have taken place during the relatively short period of storage to which these samples were subjected.

3. Chemical Method.

The British Pharmacopoeia (2) describes assay methods for the estimation of the total alkaloids in several species containing hyoscyamine and hyoscine. Lean and Ralph (7) state that these methods did

not always prove satisfactory for *Duboisia*, but Finnemore (5) found the B.P. process for *Belladonna* leaf quite satisfactory for *Duboisia* leaf containing hyoscine as the dominant alkaloid. The methods are based upon extraction with acidified alcohol and have the disadvantage that there is a large amount of co-extracted matter which has to be removed by laborious, and not always satisfactory, manipulations. To separate the alkaloids, Ralph and Willis (9), as well as Finnemore (6), used the established method of fractional alkalization. Finnemore identified the major alkaloids in such fractions by determining the melting points of the picrates crystallized from aqueous solution. The method is lengthy because the picrates often separate as a fine powder or as oily drops, which can only with great care be recrystallized to products suitable for melting point determination.

It was necessary to evolve a method for serial assay which would avoid delays due to laborious manipulations and repeated crystallizations. It was found that if chloroform, alone or in mixture with ether, is substituted for acid alcohol as the extracting menstruum, only 10 to 15 per cent. of the plant material is extracted, compared with 20 to 30 per cent. when acid alcohol is used. Furthermore, if, preliminary to the alkaloid extraction, the acidified plant material is eluted with a mixture of petroleum ether and ether, 5 to 7 per cent. of unwanted material is removed without any noticeable loss of alkaloids, and a subsequent ammoniacal chloroform-ether extract contains only 8 to 10 per cent. of plant material, most of which is insoluble in water. In samples with high alkaloid contents, the proportion of alkaloid to co-extracted matter may be as high as 1 : 4. So little pigmented matter is present that the residue, redissolved in chloroform, can be titrated directly by Vorländer's aminometric method (4, 10) for the estimation of total alkaloids. The aqueous extracts obtained from the titrated chloroform solution are colourless or only faintly yellow, and allow a clean fractionation. Difficulties encountered in obtaining well-crystallized, easily identifiable picrates were overcome by crystallizing from a two-phase system of water and ether.

The detailed assay procedure is as follows:—Three grams (or correspondingly more) of plant material are carefully moistened with 3 cc. of N/10 sulphuric acid and twice shaken for three hours with 50 cc. of ether-petroleum ether, the solvent being separated and discarded. The material is then shaken for three hours, once with 50 cc. of chloroform saturated with ammonia gas, and once with 50 cc. of ether. The combined extracts are dehydrated with sodium sulphate, concentrated to a small bulk on the water bath, and evaporated under an airblower. The residue is redissolved in 20–30 cc. of anhydrous chloroform and the total alkaloids determined by titrating with p-toluene-sulphonic acid in chloroform solution. The titrated extract is concentrated to about 15 cc. and extracted three times with 15 cc. of distilled water. The combined water-extracts are alkalized with sodium bicarbonate and extracted twice with 20 cc. of ether : hyoscine fraction. Excess of sodium carbonate is then added, and it is again extracted twice with ether : hyoscyamine fraction. A final extraction is made with chloroform : residue fraction. The latter extract is evaporated and redissolved in ether. An amount of N/10 aqueous picric acid slightly in excess of that calculated from the total alkaloid

content is added to each of the three ether extracts. On standing overnight, the picrates separate in well-shaped crystals, which are identified by their melting points, and, if necessary, and if sufficient material is available, by mixed melting points with stock alkaloid picrates, and by observing the characteristic crystal forms obtained by re-crystallizing under the microscope from dilute or absolute methyl alcohol.

A quantitative estimation of the dominant alkaloid in the fractions has not been included in the normal procedure, which has to deal with material not previously examined, and is adapted to cope with any mixture of alkaloids which can be expected. Under these conditions the fractions necessarily overlap, the predominant alkaloid may appear in all three fractions, and the residue-fraction generally collects whatever has not been previously eluted. A determination of one or other fraction by titration or by weight of the picrates would be quite unreliable. This can only be done by repeating the assay under conditions adjusted to the results of the routine examination.

In the following tables the amounts of picrates obtained in each fraction, estimated by eye observation, and compared to the expectation from the total alkaloids, are set down as follows:—

+	small amount
++	medium amount
+++	large amount

The method as described is well adapted and reliable* for the examination of large numbers of small samples; no undue delays have been experienced. About 2 per cent. of the samples examined has failed to produce crystalline picrates, but these specimens, when more thoroughly examined, proved to be very difficult with any method of extraction; the reasons for this have not yet been investigated. With a total alkaloid content of 1 per cent. or less, picrates occasionally can be obtained only if the assay is repeated with 6–10 grams of material.

The method as outlined has been used since April, 1944. Minor modifications have since been found desirable, but, in order to ensure comparability of results, they have not been incorporated in the routine method.

4. Interpretation of the Assay Results.

The alkaloids of *Duboisia* usually consist of one or two major alkaloids in association with a series of minor alkaloids and associated bases. The interpretation of the assay is confined to the relative amounts and purities of the major alkaloids present. The total alkaloid content calculated as a single alkaloid is normally too high, for there are generally certain by-alkaloids present as well as volatile and non-volatile amines which may not have been removed.

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* However, all the solvents used must be pure and free from acidic or basic substances. Many technical solvents do not fulfil these conditions and may seriously interfere with the assay.

The picrates which crystallize under the particular assay conditions show characteristic forms which are not necessarily identical with those obtained under different conditions, or from other solvents, such as alcohol.

Under the conditions of this assay, pure hyoscine picrate (melting point 187°–188°C.) crystallizes in the form of well-shaped needles; when less pure it forms branching moss-like crystals, and when very impure warts of slightly more than pinhead size. The melting points of the least typical crystals may still be within 6°–8° of the pure salt.

Pure hyoscyamine picrate (melting point 167°C.) forms small, stout, stellate prisms, often appearing as swallow-tail twins. When less pure it forms indistinct growths, needles, scales, &c., of lower (below 160°C.) melting point. Occasionally the melting points of impure forms are indistinct. The hyoscyamine fraction appears to be richer in by-alkaloids than the hyoscine fraction, and these are probably largely responsible for the aberrant crystal forms. Only the well-crystallized typical forms can be accepted as fairly pure.

Atropine (melting point 176°–177°C.) crystallizes as scales and indistinct clusters. Its presence in certain samples may be due to racemization during storage or manipulation, as it does not occur in many samples rich in hyoscyamine. It is frequently found in the hyoscine fraction, but occurs more commonly in the hyoscyamine fraction.

By-alkaloids are occasionally found as small but well-defined clusters of definite crystal form and melting point. Their non-appearance is no proof of their absence, as they sometimes appear in one duplicate of an assay but not in the other. However, they do not seem to interfere with the crystallization of the main alkaloid.

The separation of the alkaloids into fractions is not absolute, and overlapping may occur even when special efforts are made to keep the alkaloids apart. If one alkaloid is predominant, constituting, say, 70–80 per cent. of the total alkaloids, it may appear in all fractions, although in varying purity. If the second alkaloid is present in small quantities only, say, 10–20 per cent., the picrate may separate in only one fraction (often in the residue-fraction) and be badly crystallized. It is therefore not possible to consider the findings of one fraction separately; to obtain a true picture of the composition the findings in all fractions must be identified and compared. If the picrates are in sufficient quantity, well crystallized, and of sharp melting point, this presents no difficulty. If small amounts of unidentifiable precipitates are obtained, often no more can be concluded than that the specimen is less pure than others.

The residue fraction contains whatever hyoscine or hyoscyamine has not been extracted previously, together with those by-alkaloids which are sparingly soluble in ether. The picrates formed under these conditions are generally not well defined in either form or melting point. Sometimes only powdery, unidentifiable crystals separate out.

In some cases only a few crystals were obtained, and on one occasion, no crystals at all, in spite of repeated efforts and modified manipulation. This is possibly caused by "colloidal carriers" which are present in varying degrees in different samples, and which tend to inhibit precipitation. Such samples usually yield syrupy, uncrystallizable residues when preparative extracts of the alkaloids are attempted.

The relationship between the purity of the picrates obtained in the assay and the behaviour of the particular sample in preparative extraction is being investigated. Adequate amounts of the pure alkaloid were easily extracted from a series of specimens in which the assay had indicated that the alkaloid was dominant and very pure, but it appears that those samples which fail to yield well-defined picrates are difficult to process satisfactorily. The trouble seems to be caused not only by by-alkaloids, but to a great extent by non-alkaloidal impurities which are present in varying degrees.

5. Variation between Individual Trees in Natural Stands.

(i) *Duboisia myoporoides.*

The assay results for the 54 samples examined are given in Table 1. The samples are arranged in order of the latitudes of the locations from which they were collected. A line has been drawn across the table below the 46th sample to indicate the division of the species into two sections, the more southerly of which has a smaller and more pointed leaf, and a more compact habit of growth, particularly in the early stages, than has the northern type. The two sections of the species will be considered separately.

(*a*) *The Northern Section.*—The total alkaloids, expressed as a percentage of the air-dried weight of the leaf, are shown in the second column of Table 1. The figures, which vary from 0·9 to 4·0 per cent., do not appear to be related to latitude, annual rainfall, or to any obvious botanical feature of the trees, although some of the trees with the highest percentage of alkaloids seemed more vigorous than the average.

Samples from Eumundi (Q'ld.) and Yandina (Q'ld.) did not give precipitates at any stage of the routine examination. The fact that they contained less alkaloids than the remainder of the samples may have been responsible for the failure. They will not be discussed further. The other samples gave precipitates at one or other of the three stages of the routine examination. They all yielded crystalline hyoscine fractions. The quantities of the precipitates varied considerably from sample to sample, but were not closely correlated with the total alkaloids present. For example, a sample from Coolum (Q'ld.) (No. 19)* contained about 4 per cent. of alkaloids, yet only a minor quantity of picrate was precipitated from the hyoscine fraction, whereas a major amount of well crystallized picrate was obtained from a Buderim (Q'ld.) (No. 29) sample containing only 1 per cent. of alkaloids.

* The numbers in brackets are the serial reference numbers of the samples in Table 1.

TABLE 1.—THE NATURE AND AMOUNT OF ALKALOIDS IN LEAF SAMPLES FROM INDIVIDUAL TREES IN NATURAL STANDS OF *Duboisia myoporoides*.

Location.	Total Alkaloids.	Hyoscine Fraction.			Hyoscyamine Fraction.			Residue Fraction.			Dominant Alkaloid.
		Quantity.	Picrate.		Quantity.	Picrate.		Quantity.	Picrate.		
			Crystal Form.	Melting Point.		Crystal Form.	Melting Point.		Crystal Form.	Melting Point.	
	%			°C.			°C.			°C.	
1. Innisfail ..	1·1	++	Warty needles	186	+	Needles ..	188	+	Warty needles	{170 180}	Hyoscine
2. Gympie ..	1·2	++	Needles ..	187	..	..	..	Trace	..	..	Hyoscine
3. Gympie ..	1·0	++	Needles ..	186	..	..	..	Trace	..	..	Hyoscine
4. Gympie ..	1·4	++	Needles ..	187	..	..	..	..	..	..	Hyoscine
5. Gympie ..	1·5	+	Needles ..	178	+	Needles ..	173	+	Dirty needles	179	Hyoscine ?
6. Gympie ..	1·3	+	Warty needles	184	..	..	..	..	..	..	Hyoscine ?
7. Amamoor ..	1·1	++	Needles ..	187	..	..	..	++	Needles ..	{180 186}	Hyoscine ?
8. Cooroy ..	2·9	+++	Needles, balls	183	+++	Balls, feathery clumps	{170 176}	+	Balls ..	{170 178}	Hyoscine ?
9. Imbil	3·0	+++	Needles, balls	185	..	..	..	..	..	..	Hyoscine ?
10. Imbil	3·0	+++	Needles ..	187	+	Needles ..	182	Trace	..	..	Hyoscine ?
11. Imbil	2·9	+++	Needles ..	186	++	Needles ..	184	Trace	..	..	Hyoscine ?
12. Eumundi ..	0·9	..	..	..	..	..	..	..	..	..	—
13. Nandroya ..	1·7	+++	Needles ..	187	..	..	..	+	Balls ..	184	Hyoscine
14. Nandroya ..	0·9	..	..	..	..	..	..	..	..	..	—
15. Yandina ..	1·4	++	Needles ..	186	..	..	..	..	..	..	Hyoscine
16. Coolum ..	1·0	+	Long needles	187	..	..	..	..	..	..	Hyoscine
17. Coolum ..	2·2	++	Needles ..	186	..	..	..	..	..	..	Hyoscine
18. Coolum ..	1·7	+++	Needles ..	187	..	..	..	+	Balls ..	184	Hyoscine
19. Coolum ..	4·0	+	Needles, balls	184	..	..	..	..	..	..	Hyoscine ?
20. Nambour ..	1·4	+	Warty crystals	188	..	..	..	..	..	..	Hyoscine

TABLE 1.—THE NATURE AND AMOUNT OF ALKALOIDS IN LEAF SAMPLES FROM INDIVIDUAL TREES IN NATURAL STANDS OF *Duboisia myoporoides*—*continued.*

Location.	Total Alkaloids.	Hyoscine Fraction.			Hyoscyamine Fraction.			Residue Fraction.			Dominant Alkaloid.
		Quantity.	Picrate.		Quantity.	Picrate.		Quantity.	Picrate.		
			Crystal Form.	Melting Point.		Crystal Form.	Melting Point.		Crystal Form.	Melting Point.	
	%			°C.			°C.			°C.	
21. Nambour ..	2·7	+++	Warty needles	186	..	..	..	..	..	183	Hyoscine
22. Nambour ..	2·7	+++	..	188	..	..	..	..	..	181	Hyoscine
23. Wombye ..	3·6	+++	Needles ..	182	..	..	..	Trace	Needles ..	..	Hyoscine
24. Wombye ..	1·9	+	Dirty needles	183	..	..	..	..	..	..	Hyoscine
25. Wombye ..	1·3	+	Warty needles	184	..	..	..	..	..	..	Hyoscine
26. Palmwoods ..	2·8	++	Warty needles	188	..	..	..	+	Balls ..	184	Hyoscine
27. Palmwoods ..	3·1	++	Warty needles	188	..	..	..	+	Needles, balls	184	Hyoscine
28. Buderim ..	2·4	+++	Needles ..	185	..	..	..	Trace	Needles ..	..	Hyoscine
29. Buderim ..	1·0	+++	Needles ..	{184, 190}	..	..	..	..	..	..	Hyoscine
30. Redland Bay ..	1·6	+++	Needles ..	190	..	..	..	..	..	..	Hyoscine
31. Burleigh Heads	1·1	++	Needles ..	187	..	..	..	+	Needles ..	186	Hyoscine
32. Mullumbimby ..	2·0	+++	Needles ..	184	..	..	..	+	Balls ..	187	Hyoscine
33. Mullumbimby ..	1·2	+++	Needles ..	184	..	..	..	..	..	..	Hyoscine
34. Mullumbimby ..	2·7	+++	Needles ..	188	..	..	..	..	..	..	Hyoscine
35. Mullumbimby ..	2·6	+	Fuzzy balls	160	++	Long prisms	Over 245	+	Oil and fuzzy balls	175	Hyoscyamine
36. Mullumbimby ..	2·2	+	Balls ..	{164, 176}	++	Prisms, short needles	163	+	Balls ..	{166, 176}	Hyoscyamine
37. Mullumbimby ..	1·6	++	Needles, balls	184	+	Needles ..	165	+	Balls ..	{166, 176}	Hyoscine and hyoscyamine
38. Byron Bay ..	1·2	+	Needles ..	185	..	..	..	+	Needles ..	185	Hyoscine

Table 1.—The Nature and Amount of Alkaloids in Leaf Samples from Individual Trees in Natural Stands of *Duboisia myoporoides*—*continued.*

Location.	Total Alkaloids.	Hyoscine Fraction.			Hyoscyamine Fraction.			Residue Fraction.			Dominant Alkaloid.
		Quantity.	Picrate.		Quantity.	Picrate.		Quantity.	Picrate.		
			Crystal Form.	Melting Point.		Crystal Form.	Melting Point.		Crystal Form.	Melting Point.	
	%			°C.			°C.			°C.	
39. Myrtle Creek ..	1·5	++	Needles ..	186	..	..	..	++	Feathery clumps	{172, 175}	Hyoscine
40. Whiporie ..	2·1	++	Needles ..	187	..	..	..	+	Microcrystalline	187	Hyoscine
41. Grafton ..	2·3	+++	Needles ..	186	..	..	..	..	..	..	Hyoscine
42. Grafton ..	1·7	++	Coarse branching needles	184	..	..	..	..	..	..	Hyoscine
43. Coff's Harbour ..	1·4	++	Branching needles	190	..	..	..	+	Needles ..	187	Hyoscine
44. Dorrigo ..	3·4	+	Branching needles	185	++	Feathery clumps	{165, 175}	+	Needles ..	185	Hyoscine and hyoscyamine
45. Repton ..	1·2	+++	Warty needles	187	..	..	..	+	Needles ..	183	Hyoscine
46. Telegraph Point	1·0	+++	Branching needles	186	..	..	..	+	Needles ..	187	Hyoscine
47. Stroud ..	1·5	..	..	..	..	..	..	..	..	..	—
48. Stroud ..	1·4	..	..	..	+	Blades ..	163	+	Balls ..	{175, 180}	Hyoscyamine
49. St. Albans ..	1·0	..	..	..	..	..	..	..	..	..	—
50. St. Albans ..	1·6	..	..	..	++	Prisms ..	165	+	Prisms, blades	162	Hyoscyamine
51. St. Albans ..	2·0	..	..	..	..	..	..	..	..	..	—
52. Gosford ..	2·7	..	..	..	+++	Prisms ..	167	+	Solid clusters	180	Hyoscyamine
53. Gosford ..	2·3	..	..	..	++	Prisms ..	162	..	..	..	Hyoscyamine
54. Gosford ..	1·5	..	..	..	+	Prisms ..	165	..	..	..	Hyoscyamine

The melting points of the picrates varied considerably. However, 38 lay within the 182°–188°C. range, and were identified as hyoscine picrate of varying purity. A further three samples (Nos. 29, 30, 43) gave precipitates melting at 190°C., which were also identified as hyoscine picrate.† The remaining samples, two of which were from near Mullumbimby (N.S.W.) (Nos. 35, 36), and the third, from Gympie (Q'ld.) (No. 5), yielded picrates melting at 160°C., 164°–176°C., 178°C., respectively. The first two consist mainly of impure hyoscyamine, but the identity of the third could not be determined as the quantity was insufficient. It may possibly be atropine.

Thus 41 of the 44 hyoscine fractions consisted of hyoscine of varying degrees of purity, two of impure hyoscyamine, and one was not identified.

Nine samples gave hyoscyamine fractions. Three of these consisted of reasonably pure hyoscine, and will not be discussed further. The first of the Mullumbimby trees which did not yield hyoscine in the hyoscine fraction gave a hyoscyamine fraction precipitate melting at above 245°C., which has been identified as tropine picrate. The second Mullumbimby tree gave a fairly pure hyoscyamine picrate, and the Gympie, an unidentified crystalline precipitate very similar to that which appeared in the hyoscine fraction. The remaining samples which gave hyoscyamine fractions were from Cooroy (Q'ld.) (No. 8), Mullumbimby (N.S.W.) (No. 37), and Dorrigo (N.S.W.) (No. 44), the fractions consisting, respectively, of impure atropine, hyoscyamine, and a mixture of impure hyoscyamine and impure atropine.

Residue fractions were recorded in 25 samples. The precipitates usually resembled those obtained from the earlier fractions, but occasionally different crystal forms having appreciably lower melting points appeared, as, for example, the sample from Myrtle Creek (N.S.W.) (No. 39), which gave typical hyoscine picrate, no hyoscyamine fraction, and substantial quantities of feathery clumps melting at 172°–175°C., in the residue fraction.

Thus, of the 46 samples from the northern section of *D. myoporoides*. 38 contained hyoscine of varying purity, three contained both hyoscine and hyoscyamine and/or atropine, one hyoscyamine and atropine, and one hyoscyamine and tropine.* A disproportionate number of atypical trees has been found in the Mullumbimby (N.S.W.) area, but the presence of hyoscyamine is not restricted to samples from there.

Of the 46 trees, 12 yielded more than 2·5 per cent. of alkaloids, and 6 of these gave adequate quantities of hyoscine picrate of good crystal form and melting point. When these samples were subjected to preparative extraction in the laboratory, over 80 per cent. of the total alkaloids were recovered as pure recrystallized hyoscine hydrobromide, whereas others yielded only a small proportion of pure alkaloid. The

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† A mixture with stock hyoscine picrate melted at 177°–188°C. The higher melting points may have been due to overheating or similar cause.

* Tropine is formed from hyoscyamine by hydrolysis. It is not known whether this has occurred inside the plant or during subsequent manipulations, but the latter explanation seems the more likely, as the high melting point picrate was not obtained when the assay was repeated some weeks later.

presence of certain non-alkaloidal material is probably as important a criterion for selection as is the percentage of total alkaloids or the nature and amount of by-alkaloids.

(*b*) *The Southern Section.*—Only eight samples of this section have been assayed. The total alkaloids ranged from 1·0 to 2·7 per cent. Three samples failed to give precipitates at any stage of the routine examination, and crystals appeared very slowly in several of the others. It is suggested that colloidal carriers may be responsible for the difficulties experienced in obtaining precipitates in the group. None yielded hyoscine fractions, and the amounts of hyoscyamine fraction were small in four of the five cases in which precipitates were obtained. However, the major alkaloid found in all cases was hyoscyamine.

The numbers of trees sampled are too few from which to draw definite conclusions regarding the nature of the alkaloids in the group, but the data available suggest that hyoscyamine is the major alkaloid present.

(ii) *Duboisia Leichhardtii.*

The assay results for the 28 samples examined are shown in Table 2. The species is now confined to a small area in south-eastern Queensland, and as a result the samples were obtained from relatively few centres. The location "South Nanango" includes material collected within ten miles of the town of that name. The total alkaloids range from 0·8 to 3·7 per cent. of the dry weight of the leaves. The variation does not appear to be associated with any obvious environmental or botanical feature.

The 28 samples all yielded crystalline precipitates at one or more stages of the routine examination, and 22 gave hyoscine fractions. Of these, 8 consisted principally of hyoscine, 13 of hyoscyamine and/or atropine, and 1 was not identified, but crystallized as balls and melted at 215°C.; it may possibly have been nor-hyoscyamine.

In all, 24 samples gave hyoscyamine fractions. Two of these consisted mainly of hyoscine, and the remainder, of hyoscyamine of varying purity.

The residue fractions were, in many cases, different in character to the preceding fractions. For example, a South Nanango (Q'ld.) (No. 14) tree, which contained hyoscine and hyoscyamine of average purity, gave a residue fraction melting at 210°C.* The sample from Mannuem Creek (Q'ld.) (No. 8) contained a large amount of fairly pure hyoscyamine, yet balls melting at 180°C. (hyoscine) crystallized from the residue. Minor alkaloids may be responsible for these divergences.

As in *D. myoporoides*, the amounts of the precipitates did not always correspond to the total alkaloids. However, of the ten trees containing more than 2·5 per cent. alkaloids, six yielded equivalent quantities of hyoscyamine picrate of good crystal form and melting point.

* This was neither methylamine (m.p. 206°C.) nor nicotine (m.p. 223°C.) and was possibly nor-hyoscyamine.

Thus, of the 28 trees examined, 5 contained hyoscine, 19 hyocyamine of varying purity, and 4 appreciable amounts of both hyoscine and hyoscyamine. It is clear that the trees comprising natural stands of the species differ in total alkaloids, in the nature and purity of the dominant alkaloid present, and in the amount of the major alkaloids crystallizing under the conditions of the assay.

6. Changes in the Alkaloids in Natural Stands and of Plantation Material Grown in Different Habitats.

(i) *Samples from Individual Trees at Different Times.*

Four trees of *D. Leichhardtii* were sampled in November, 1943, and again in October, 1944, i.e., during the flowering-fruiting season in successive years. Samples were collected from three trees of *D. myoporoides* during October-November, 1942, and in December-March, 1945. The results of the assays of these samples are given in detail in Table 3.*

None of the *D. Leichhardtii* trees contained hyoscine at the 1943 sampling, yet in 1944 large amounts of that alkaloid were present in two of the trees, and a smaller amount in a third. The quantity of hyoscyamine remained fairly constant in three trees, but decreased considerably in the fourth. The *D. myoporoides* tree that contained both hyoscine and hyoscyamine on the first occasion, yielded mainly hyoscine on the second. The two that contained hyoscine and hyoscyamine respectively as the major alkaloid, did not change materially.

It is evident that considerable changes took place in four of the seven trees. The behaviour of the *D. Leichhardtii* trees indicates that conditions in 1944 were apparently more favourable for the development of hyoscine in that species than they were in 1943.

The quantity of alkaloids in the *D. myoporoides* trees remained fairly constant, but all four trees of *D. Leichhardtii* increased in total alkaloids during the twelve months.

(ii) *Samples from Plantation Material in Different Environments at Different Times.*

Material of both species has been cultivated at Nambour (Q'ld.) and Canberra (A.C.T.). The former is situated in a high rainfall sub-tropical coastal zone, and the latter in a highland temperate region with a large diurnal temperature range, and an average annual rainfall of about 20 inches.

Leaf samples were taken at intervals from several progenies, including seed lines of northern *D. myoporoides* and of *D. Leichhardtii* which were growing under comparable conditions at both centres.

* In the assay method used, hyoscine is easily identified, even if present in very small amounts, so that its presence or absence in a sample is usually quite definite. However, hyoscyamine only appears as well-crystallized prisms if the alkaloid is very pure or present in large amount. Otherwise, it may be badly crystallized and have a low melting point owing to mixture with impurities. Mixed melting point determinations with stock hyoscyamine picrate have proved that such small quantities of low melting point precipitates, almost always consist of impure hyoscyamine and/or atropine picrate. If hyoscyamine has been determined in one of several samples from the same plot, then it may safely be assumed that other low melting point second fractions in the series also contain impure hyoscyamine.

TABLE 2.—THE NATURE AND AMOUNT OF ALKALOIDS IN LEAF SAMPLES FROM INDIVIDUAL TREES IN NATURAL STANDS OF *Duboisia Leichhardtii*.

Location.	Total Alkaloids.	Hyoscine Fraction.			Hyoscyamine Fraction.			Residue Fraction.			Dominant Alkaloid.
		Quantity.	Picrate.		Quantity.	Picrate.		Quantity.	Picrate.		
			Crystal Form.	Melting Point.		Crystal Form.	Melting Point.		Crystal Form.	Melting Point.	
	%			°C.			°C.			°C.	
1. Proston ..	2·6	+++	Needles ..	184	++	Needles ..	186	++	Balls ..	166	Hyoscine
2. Proston ..	3·7	..	..	..	..	..	..	+	Balls ..	176	*
3. Proston ..	2·9	+++	Needles ..	190	..	..	..	..	..	..	Hyoscine
4. Proston ..	1·8	+++	Warty needles ..	188	..	..	..	+	Balls ..	180–185	Hyoscine
5. Proston ..	3·2	++	Balls ..	160	++	Prisms ..	167	..	..	..	Hyoscyamine
6. Wondai ..	2·3	+++	Needles ..	186	+++	Needles ..	182	+	Balls ..	168	Hyoscine
7. Wondai ..	2·1	++	Balls ..	162–176	+++	Prisms ..	164	..	..	..	Hyoscyamine
8. Mannuem Creek	2·2	..	..	..	+++	Prisms ..	164	+	Balls ..	180	Hyoscyamine
9. South Nanango	3·5	+	Mossy ..	177	+++	Prisms ..	163	++	Prisms ..	158	Hyoscyamine
10. South Nanango	1·9	+	Mossy ..	180	+++	Prisms ..	162	Trace	..	..	Hyoscyamine
11. South Nanango	0·8	+	Mossy ..	184	..	..	..	Trace	..	..	Hyoscine
12. South Nanango	2·2	++	Warty needles	186	+	Balls ..	164	+	Balls ..	178	Hyoscine and hyoscyamine
13. South Nanango	2·3	+	Balls ..	215	+++	Prisms ..	161	+	Prisms ..	159	Hyoscyamine
14. South Nanango	1·4	++	Needles balls	192	+++	Prisms ..	160	+	..	210	Hyoscine and hyoscyamine
15. South Nanango	2·9	..	..	..	+++	Prisms ..	161	..	..	..	Hyoscyamine
16. South Nanango	2·9	+	..	172	+++	Prisms ..	161	+	..	168	Hyoscyamine
17. South Nanango	2·0	Trace	..	..	++	Prisms ..	163	+	Needles, balls	160	Hyoscyamine
18. South Nanango	3·1	++	Warty needles balls	158	+++	Prisms ..	161	+	..	176	Hyoscyamine
19. South Nanango	2·7	..	..	..	+++	Prisms ..	160	+	Prisms ..	160	Hyoscyamine

* Was not determined owing to the small amount of precipitate.

TABLE 2.—THE NATURE AND AMOUNT OF ALKALOIDS IN LEAF SAMPLES FROM INDIVIDUAL TREES IN NATURAL STANDS OF *Duboisia Leichhardtii*—*continued.*

Location.	Total Alkaloids.	Hyoscine Fraction.			Hyoscyamine Fraction.			Residue Fraction.			Dominant Alkaloid.
		Quantity.	Picrate.		Quantity.	Picrate.		Quantity.	Picrate.		
			Crystal Form.	Melting Point.		Crystal Form.	Melting Point.		Crystal Form.	Melting Point.	
	%			°C.			°C.			°C.	
20. South Nanango	1·8	+	Balls ..	177	+	Balls, plates	156	Trace	..	..	Hyoscyamine
21. South Nanango	2·3	..	..	..	+++	Prisms ..	162	Trace	..	..	Hyoscyamine
22. South Nanango	2·6	+	Plates ..	160	+++	Prisms ..	162	..	..	..	Hyoscyamine
23. South Nanango	1·7	+	Balls ..	174	+	Plates ..	158	+	Balls ..	178	Hyoscyamine
24. South Nanango	2·8	++	Coarse branching	175	++	Plates, prisms	160	Trace	Balls ..	172	Hyoscyamine
25. South Nanango	2·2	+	Balls, plates	{158 166}	+	Prisms, plates	{157 160}	Trace	Needles, balls	188	Hyoscyamine
26. South Nanango	1·9	+	Needles ..	160	+++	Prisms ..	163	++	Prisms, balls	{160 170}	Hyoscyamine
27. South Nanango	1·6	..	..	..	+++	Prisms ..	163	Trace	Balls ..	..	Hyoscyamine
28. South Nanango	1·7	+	Fuzzy balls	172	+	Plates, micro-crystals	{156 159}	+	Balls ..	172	Hyoscyamine

TABLE 3.—THE NATURE AND AMOUNT OF THE ALKALOIDS OF INDIVIDUAL TREES OF *Duboisia* SPP. AT DIFFERENT TIMES.

Location.	Time of Sampling.	Species.	Total Alkaloids.	Hyoscine Fraction.			Hyoscyamine Fraction.			Residue Fraction.		
				Quantity.	Picrate.		Quantity.	Picrate.		Quantity.	Picrate.	
					Crystal Form.	Melting Point.		Crystal Form.	Melting Point.		Crystal Form.	Melting Point.
			%			°C.			°C.			°C.
Mullumbimby	Nov., 1942	*D. myoporoides*	1·6	++	Needles, balls	184	+	Needles	165	+	Balls ..	{166 176}
	Mar., 1945	,, ,,	1·5	++	Needles ..	185	..	..	..	+	Needles, balls	158
Mullumbimby	Nov., 1942	*D. myoporoides*	2·2	++	Balls ..	{164* 176}	++	Prisms, short needles	163	+	Balls ..	{166 176}
	Mar., 1945	,, ,,	2·2	..	.. ..	..	+	Fuzzy balls	169	+	Oil, balls	164
Nambour ..	Oct., 1942	*D. myoporoides*	2·7	+++	Warty needles	186	..	..	..	..	..	183
	Dec., 1944	,, ,,	3·1	+++	Small needles	187	..	..	..	+	Balls ..	182
South Nanango	Nov., 1943	*D. Leichhardtii*	2·9	..	.. ..	..	+++	Prisms	161	..	..	..
	Oct., 1944	,, ,,	3·8	+	Balls ..	184	+++	Prisms	164	..	..	..
South Nanango	Nov., 1943	*D. Leichhardtii*	2·3	..	.. ..	..	+++	Prisms	162	Trace	..	..
	Oct., 1944	,, ,,	2·8	+++	Branching needles	185	+++	Prisms	165	..	..	..
South Nanango	Nov., 1943	*D. Leichhardtii*	2·8	++	Coarse branching needles	175*	++	Plates, prisms	160	Trace	Balls ..	172
	Oct., 1944	,, ,,	3·7	++	Long needles	160*	++	Prisms, plates	165	..	..	..
South Nanango	Nov., 1943	*D. Leichhardtii*	1·6	..	.. ..	..	+++	Prisms	163	Trace	Balls ..	..
	Oct., 1944	,, ,,	1·9	+++	Needles ..	181	+	Balls ..	{173 176}	..	..	..

* Hyoscyamine/atropine.

The relative amounts of hyoscine and hyoscyamine precipitated as picrates under the condition of the assay, at successive samplings of lines grown both at Nambour and Canberra, are illustrated graphically in Fig 1. The scale is arranged on a 0 to 4 basis, the

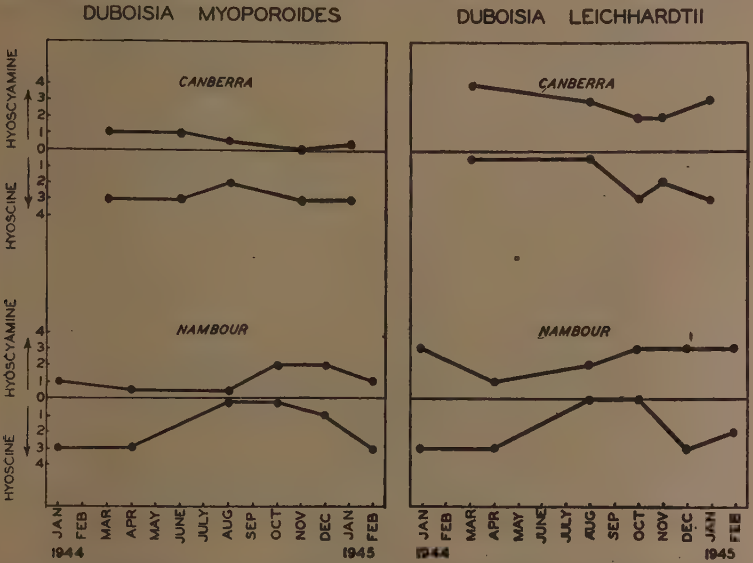

FIG. 1.—The variation in the amount of hyoscine and hyoscyamine in young trees of *Duboisia myoporoides* and *Duboisia Leichhardtii* at Canberra (A.C.T.) and Nambour (Q'ld.).

larger figure representing the greatest amount of well-crystallized picrate. The hyoscyamine and hyoscine are plotted above and below a zero reference line, so that the difference between the two curves represents the total picrates. The quantity and nature of the alkaloids fluctuated considerably at both places, but the range of variation at each was approximately the same. Hyoscine and hyoscyamine in turn became the dominant alkaloid in *D. Leichhardtii* at both Nambour and Canberra, and in *D. myoporoides* at Nambour, whilst the *D. myoporoides* grown at Canberra contained variable amounts of hyoscine, often accompanied by hyoscyamine. The most consistent feature is the tendency for a minimum quantity of precipitated picrates at mid-winter and a maximum at mid-summer. The fluctuations in the hyoscyamine/hyoscine ratio are not concordant, either between places or between species. Hyoscine seems to be somewhat more sensitive to changes in environmental conditions than does hyoscyamine, although there is evidence that the alkaloids are, at least to some extent, complementary. However, this may tend to be masked by fluctuations in total alkaloids, and in the amount of non-alkaloidal substances inhibiting precipitation which are present.

The maximum variation in the nature of the alkaloids is shown in detail in Table 4. Hyoscine was the dominant alkaloid in both *D. Leichhardtii* and *D. myoporoides* at Nambour in April, yet by

TABLE 4.—THE MAXIMUM DIFFERENCE IN THE NATURE OF THE ALKALOIDS IN *Duboisia* SPP. CULTIVATED AT NAMBOUR (Q'LD) AND CANBERRA (A.C.T.).

Location.	Species.	Time of Sampling.	Hyoscine Fraction.			Hyoscyamine Fraction.			Residue Fraction.		
			Quantity.	Picrate.		Quantity.	Picrate.		Quantity.	Picrate.	
				Crystal Form.	Melting Point.		Crystal Form.	Melting Point.		Crystal Form.	Melting Point.
					°C.			°C.			°C.
Nambour ..	*D. Leichhardtii* ..	Oct., 1944	..	.. ..	..	+++	Prisms ..	162	..	.. ..	..
		Apr., 1944	+++	Needles ..	181	+	Balls, irregular prisms	158	+	Balls ..	175
Canberra ..	*D. Leichhardtii* ..	Aug., 1944	+	Branching needles	175*	+++	Prisms ..	{160, 163}	++	Plates ..	..
		Oct., 1944	+++	Branching needles	188	++	Prisms ..	164	+	Prisms, balls	168
Nambour ..	*D. myoporoides* ..	Oct., 1944	++	Balls ..	{172, 178}	++	Prisms ..	164	..	.. ..	..
		Apr., 1944	+++	Needles ..	184	+	Balls ..	170	+	Balls, needles	181
Canberra ..	*D. myoporoides* ..	May, 1943	++	Needles, balls	184	++	Prisms, balls	168	++	Balls ..	{172, 179}
		Nov., 1944	+++	Branching needles	188	..	.. ..	..	..	.. ..	..

* Identity doubtful.

October it had disappeared. Hyoscine was not present in leaf of *D. Leichhardtii* collected at Canberra in August, but by October it was the dominant alkaloid. Hyoscyamine and hyoscine were present in approximately equal amounts in *D. myoporoides* at Canberra in May 1943, yet only hyoscine was found in November 1944.

The percentage of total alkaloids at the different times of sampling is shown in Table 5. The average value for *D. Leichhardtii* at Canberra was higher than at Nambour, but the values for *D. myoporoides* were not appreciably different. There was moderate variation at each centre, one considerable fluctation, however, being recorded at Nambour between April and August, when the total alkaloids in leaf of *D. myoporoides* decreased from 3·1 to 1·8 per cent., and in *D. Leichhardtii* from 2·8 to 1·7 per cent.

TABLE 5.—THE PERCENTAGE OF TOTAL ALKALOIDS IN *Duboisia* SPP. AT NAMBOUR (Q'LD.) AND CANBERRA (A.C.T.) WHEN SAMPLED AT DIFFERENT TIMES OF THE YEAR.

Time of Collection of Leaf.	Percentage Total Alkaloids in Air-dried Leaf.			
	D. Leichhardtii.		*D. myoporoides.*	
	Canberra.	Nambour.	Canberra.	Nambour.
Jan., 1944	..	2·5	..	2·7
Mar., 1944	4·3	..	2·7	..
Apr., 1944	..	2·7	..	3·1
June, 1944	..	..	3·0	..
Aug., 1944	3·0	1·7	3·0	1·8
Oct., 1944	3·9	3·1	..	3·0
Nov., 1944	2·2	..	2·6	..
Dec., 1944	..	3·2	..	3·3
Jan., 1945	3·7	..	2·3	..
Feb., 1945	..	2·9	..	3·8
Mean Value	3·4	2·7	2·7	2·8

Although data are available for other seed lines, some of which have been sampled periodically for two years, it has not yet been possible to trace a consistent pattern of variation in either the amount or the nature of the alkaloids. However, *D. myoporoides* was a good source of hyoscine, and *D. Leichhardtii,* of hyoscyamine, at mid-summer during two successive seasons at both Canberra and Nambour.

Experiments to discover the cause of the variations are continuing, for it is clear that they must be explained and controlled if *Duboisia* is to prove a permanent and reliable source of hyoscine and hyoscyamine.

7. Conclusions.

Trees containing hyoscine and hyoscyamine in various proportions, and others in which the alkaloids were present singly, were found in natural stands of both *Duboisia myoporoides* and *Duboisia Leichhardtii.*

A small proportion of trees also contained appreciable quantities of other alkaloids, such as atropine and nor-hyoscyamine. Minor alkaloids were found in most of the trees examined, and may possibly always accompany the major alkaloids.

The frequency distribution of the population types evidently controls the alkaloid content of aggregate samples. It would appear that the population balance favours hyoscine in the northern section of *D. myoporoides*, and hyoscyamine in *D. Leichhardtii* and the southern section of *D. myoporoides*.

There was considerable variation in the amount of total alkaloids in the individual trees of each species, and also in the proportion of alkaloids which appeared as crystalline picrates under the conditions of the assay. There is evidence that the latter character is correlated with the ease with which preparative extracts of the pure alkaloids may be made, and its variability is possibly due to the presence of greater or lesser amounts of as yet undefined non-alkaloidal bodies or colloidal carriers, which interfere with precipitation.

Efforts to correlate the various differences observed between individual trees, with location, soil, climate, or obvious botanical features of the trees, have not yet been successful.

The nature and amount of alkaloids in individual trees of natural stands are not constant, but apparently vary from time to time and from year to year. Nevertheless, it seems that, on the average, the alkaloid balance in natural stands is very similar to that disclosed by the individual tree survey. This suggestion is supported by unpublished assay results for a number of aggregate samples collected at different times, and by commercial extraction experience.

Considerable changes in the nature and quantity of the alkaloids also took place from time to time in young plantation material, the major alkaloid changing from hyoscine to hyoscyamine within as short a time as three months. Although under certain conditions hyoscine was the major alkaloid in *D. Leichhardtii*, and hyoscyamine in northern *D. myoporoides*, the average composition of the alkaloids in young plantation grown leaf was much the same as that found in natural stands. The proportion of the alkaloids appearing as crystalline picrates varied during the year, but the variation was not so great as that observed among individual trees in natural stands.

The variation in the nature and amount of the alkaloids was more pronounced at Nambour (Q'ld.) than Canberra (A.C.T.), although the same type of change was evident at each. In two seasons at both Nambour and Canberra mid-summer appeared to be a suitable time for the collection of hyoscine-bearing leaf of northern *D. myoporoides* and of hyoscyamine-bearing leaf of *D. Leichhardtii*.

The evidence available at this stage of the investigation suggests that both heredity and environment play a part in determining the nature of the alkaloids in *Duboisia*, but that under certain conditions environment may be the controlling factor. Experiments are continuing, with the object, amongst others, of determining the cause of the environmental variations observed, and of defining the limits within which the quantity and nature of the alkaloids are inherited, both within and between the species.

8. Acknowledgments.

The authors wish to acknowledge their indebtedness to the Queensland Sub-Department of Forestry, whose assistance in collecting samples has been invaluable, to the Queensland Department of Agriculture and Stock for the facilities and help provided at Nambour, especially by Mr. H. M. Groszmann, who took a major part in the early stages of the investigation, to the New South Wales Forestry Department for the collection of material in that State, and to Professor R. D. Wright of the Department of Physiology, University of Melbourne, for providing facilities and help on the chemical side.

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Unavailability of Plant Food and Take-all of Wheat.

By H. R. Angell, Ph.D.

Erratum.

An error occurred on page 44 of this article which appeared in the February, 1945, issue of the *Journal*. In line 8, the statement "weighed more and produced more grain" should read "weighed less and produced less grain."

Service Tests of Fluarized Karri Rail Sleepers in Western Australia.

*By N. Tamblyn, M.Sc.(Agric.)**

Summary.

In 1929 service tests were commenced in Western Australia to determine the preservative value of the fluarizing process for the treatment of green karri (*Eucalyptus diversicolor* F.v.M.) rail sleepers. The tests were a co-operative project between the Division of Forest Products, the Western Australian Government Railways, and the Western Australian Forests Department.

The fluarizing process was a hot diffusion treatment developed and patented by the Western Australian Forests Department during the period 1924-26 as an alternative to the previously used powellizing process. Treatment consisted of boiling the green sleepers in the preservative for ten hours followed by a cooling period of 36 hours before removal from the solution. The preservative was an aqueous solution of sodium fluoride, arsenious oxide, and sodium dinitrophenate.

In the test approximately 3,000 fluarized karri sleepers were installed in 22 test sections in the Western Australian Government Railways system. The average service life of all fluarized karri sleepers in the test is now estimated at 13·0 years, ranging from 10·1–17·8 years in the 22 test sections. Untreated karri sleepers installed as controls in one test section only, gave an average service life of 6·3 years compared with an estimated life in excess of twenty years for untreated jarrah controls in the same section.

Treated sleepers failed principally from decay associated occasionally with termite attack. In some sections where decay hazard was low, mechanical failure also assumed importance in the later years of test. It is considered that the weakness of the treatment lay in the shallow penetration of the preservative and the susceptibility of the treated sleepers to end-splitting, thus exposing the inner untreated wood to fungal or termite attack.

1. Introduction.

Karri (*Eucalyptus diversicolor* F.v.M.) is a Western Australian timber similar to jarrah in the appearance and density of the converted timber, but with lower durability in contact with the ground where decay or termite hazard exists. In common with other eucalypt timbers, the truewood of karri has proved refractory to pressure treatment with preservatives but may be penetrated slowly by diffusion of water-soluble chemicals into the green timber. This method of treatment by diffusion was the basis of the powellizing process which was used extensively in Western Australia in the period 1910-26 for the treatment of green karri sleepers. In this process the green timber was boiled in open vats for several hours in a solution containing 6-8 per cent. molasses and 1-2 per cent. arsenious oxide, and then slowly cooled in the preservative. While powellized karri sleepers have given excellent results in certain arid areas and generally have proved resistant to termite attack, the treatment gave less protection where moderate or severe decay hazard existed. Modification of the process to improve its preservative value for wetter areas was therefore considered necessary, and with this object experimental work was undertaken in 1924-26 by the Wood Technology Investigation Committee of the Western Australian Forests Department.

* An officer of the Division of Forest Products.

As a result a treatment was developed in which sodium fluoride and sodium dinitrophenate was substituted for the molasses, the new preservative having approximately the following composition by weight:—

Sodium fluoride (95-97 per cent. purity)	3·7 per cent.
Sodium dinitrophenate (100 per cent.) ..	0·2 per cent.
Arsenious oxide (95 per cent.) ..	1·0 per cent.
Soda ash	0·2 per cent.
Water	94·9 per cent.

This treatment was patented by the Western Australian Forests Department, and the name "Fluarizing process" coined to describe its preservative value, which was due mainly to the fluorine and arsenic.

The treating plant which was used for both fluarizing and powellizing was located at the State Saw Mill, Pemberton, in the karri forest area, and consisted of six steam-heated treating vats together with accessory tanks and boilers. Each vat was approximately 90 feet long, 10 feet wide, and 7 feet deep, with end doors to permit entry of the trucks on which the green timber was stacked with separating strips to permit free access of solution during treatment. Sleepers were treated by boiling for ten hours in the preservative followed by a slow cooling period of 36 hours before removal from the solution.

Karri sleepers thus treated were not completely penetrated, and it is probable that effective treatment was limited to a depth of ¼-½ inch on the faces and edges with somewhat deeper end-grain penetration. The following analyses for sodium fluoride and arsenic were made by J. E. Cummins, when connected with the development of the process as an officer of the Western Australian Forests Department. Specimens were prepared by planing off and discarding the outer 1/16 inch and then sampling in ¼-in. intervals.

	Per cent. NaF.	Per cent. As_2O_3.
First ¼ inch	0·60 ..	0·015
Second ¼ inch	0·15 ..	0·007
Third ¼ inch	0·03 ..	0·005

The total absorption of preservative for sleepers 7 ft. x 9 in. x 4½ in. averaged approximately 0·08 lb. of dry salt per cubic foot of wood. The treatment cost at the plant was low, being approximately 16s. per load of 50 cubic feet, this figure including labour and chemicals but not certain costs of administration and sales organization.

2. Installation of Tests.

The fluarizing treatment was commenced in 1926 without field test of its preservative value, the originators relying on overseas tests which had demonstrated the toxicity of the salts employed. In 1929, however, the Division of Forests Products approached the Western Australian Government Railways and the Forests Department to co-operate in installing a service test of fluarized karri sleepers. Suitable arrangements were made, and in October and November, 1929, about 3,000 fluarized karri sleepers were installed in 22 test sections in four different localities in Western Australia. An unfortunate defect of the test, which was beyond the control of the Division, was the lack of untreated control sleepers at the time of the original installation. To some extent this

was overcome in 1930 by laying a control section of untreated karri sleepers in one test locality (Bowelling-Wagin), and later again in 1934 by inclusion of untreated jarrah sleepers in the same locality.

The four localities selected for test were representative of different types of service environment which may be described as follows:—

(i) *Eastern Goldfields Railway:*—237-342 *miles from Perth.*
Average rainfall—10·5 inches at Southern Cross (241 m.).
9·7 inches at Kalgoorlie (380 m.).
Decay hazard relatively low, termite hazard severe.

(ii) *Bowelling-Wagin line:*—180-200 *miles from Perth.*
Average rainfall—28·4 inches at Bowelling (147 m.).
17·7 inches at Wagin (210 m.).
Decay hazard fairly severe, termite hazard low.

(iii) *Bolgart-Calingiri line:*—98-109 *miles from Perth.*
Average rainfall—18·5 inches at Bolgart (89 m.).
17·7 inches at Calingiri (104 m.).
Decay and termite hazard moderate.

(iv) *Wyalcatchem-Bencubbin line:*—167-182 *miles from Perth.*
Average rainfall—13·5 inches at Wyalcatchem (140 m.).
12·1 inches at Bencubbin (192 m.).
Decay hazard relatively low, termite hazard moderate.

The decay hazard was probably accentuated in the last three localities, where the fluarized karri test sleepers were laid in the road bed and ballast from which rotten powellized karri sleepers had been removed. The above assessment of termite hazard refers to the test localities generally, without consideration of any local deterrent effect due to vibration of sleepers by rail traffic.

3. Inspections.

With the exception of sections on the Eastern Goldfield's Railway, all test sleepers were numbered with zinc-coated numeral nails so that an inspection record could be kept showing the progressive deterioration of each sleeper until its date of renewal. Routine annual maintenance inspections of all sections were made by the Western Australian Government Railways and returns were forwarded to the Division of Forest Products giving the numerals of all sleepers renewed. In addition to these maintenance records, officers of the Division made periodic inspections in which the ends of all test sleepers were opened up to the rail seat by removal of ballast, and the condition of each sleeper then recorded with reference to severity of decay or termite attack. The mechanical condition was also recorded where splitting was a contributory cause of failure. These inspections were made with the assistance of the engineer or permanent way inspector of the Western Australian Government Railways responsible for the section. The test sections on the Eastern Goldfield's Railway were not inspected by officers of the Division, records being compiled entirely from data supplied by the Western Australian Government Railways.

As less than 30 per cent. of the 2,961 fluarized karri sleepers installed in 1929 were still in service after the 1944 inspection, the remaining service life of all test sleepers still in the track was estimated to permit issue of a final report. Estimation of remaining service life was made

by the engineer or permanent way inspector responsible for the maintenance of the section and was checked by the Senior Timber Inspector of the Western Australian Forests Department (Mr. L. N. Weston) and the author.

4. Results.

The results are presented as service and inspection records in Tables 1-3. The service records are compiled from annual reports supplied by the Western Australian Government Railways, while the inspection records summarize the condition of sleepers at each inspection by officers of the Division.

TABLE 1.—SERVICE RECORDS OF FLUARIZED KARRI TEST SLEEPERS ON EASTERN GOLDFIELDS LINE, WESTERN AUSTRALIA.

Mileage from Perth.	Date Installed (1929).	No. of Sleepers Installed.	Renewals (all causes) for years ending June 30.							Average Service Life (years).
			1937.	1938.	1939.	1940.	1941.	1942.	1943.	
237	Oct.	73	..	..	20	..	5	..	48	11·9
241	,,	63	3	..	1	All sleepers removed June, 1940, owing to construction of deviation.				..
255	,,	44	..	..	..	44	..	..	..	10·1
272	,,	57	..	..	22	..	7	1	27	11·3
282	,,	57	..	..	..	..	15	..	42	12·6
288	,,	56	..	..	..	..	..	56	..	12·1
295	,,	57	..	..	..	..	17	40	..	11·8
310	,,	57	3	..	5	..	..	49	..	11·6
313	,,	59	..	..	5	1	53	..	..	10·9
326	Dec.	56	..	..	..	..	56	..	..	11·0
327	,,	57	..	..	10	1	46	..	..	10·7
340	Nov.	57	..	..	..	..	..	..	57	13·1
342	,,	57	..	..	..	..	57	..	..	11·1

5. Discussion of Results.

(i) *Eastern Goldfield's Railway.*

As test sections in this locality were not inspected by officers of the Division of Forest Products, a detailed analysis of causes of failure cannot be made. It is, however, stated in reports by the District Engineer for Kalgoorlie that failure was due principally to decay and "spike kill." Many of the sleepers were rebored and renewals were not heavy until the last years of service. According to reports, termite attack was slight despite the fact that termite hazard is severe in the Southern Cross-Kalgoorlie area. In fence post tests installed near Southern Cross, untreated posts of several timber species were completely destroyed by termites in less than three years. However, in the absence of untreated karri sleepers as controls the interpretation of this result is not clear. Termite attack occurred in the Bolgart-Calingiri and Wyalcatchem-Bencubbin tests where rail traffic is relatively infrequent, and it is possible that the effect of vibration from heavy traffic on the Eastern Goldfields line was an important factor in discouraging termite attack.

As decay hazard is relatively low in this locality the average service life of 11·6 years for the 687 sleepers in test was below expectation. This figure is lower than recorded for the sections at Bowelling-Wagin (14·0 years), Bolgart-Calingiri (13·4 years) and Wyalcatchem-Bencubbin (13·6 years) and is less than half the life obtained from powellized karri on the Commonwealth Railway east of Kalgoorlie. In comparison with other test sections, the faster and heavier traffic, the more stringent maintenance, and the severe summer climatic conditions conducive to splitting and "brooming" (i.e., fraying and lifting of the exposed surface) are possible reasons for the shorter service life.

(ii) *Bowelling-Wagin line.*

In this locality, where comparative figures are available for the untreated karri and jarrah controls, the preservative value of the fluarizing process can be interpreted more satisfactorily than in the other localities where the absence of controls was a serious defect.

Unfortunately, the 107 untreated karri sleepers were laid in one section continuous with one set of fluarized karri sleepers, but 5-11 miles distant from the other treated groups in this locality. The comparison is thus valid only for the one adjacent treated group. The 107 untreated karri sleepers gave an average service life of 6·3 years, compared with 12·3 years for the adjacent length of 140 fluarized sleepers. These untreated sleepers failed rapidly from decay and slight termite attack, the first renewals being made approximately three and a half years after installation. In this and other test localities, the presence of fruiting bodies of the wood destroying fungus *Trametes lilacino-gilva* Berk. on both treated and untreated karri sleepers indicated that this fungus was responsible largely for decay failure. From many observations karri is known to be particularly susceptible to attack by this fungus, which has been recorded from all Australian States.

The average service life of the 750 fluarized sleepers was 14·0 years, ranging from 12·3-17·8 years for the four different lengths, the first renewals being made after approximately six years' service. Decay was the principal cause of failure, termite attack being restricted to untreated sleepers. Splitting was indirectly an important cause of failure, as many cases of internal decay were associated with deep end-splits extending to the rail seat. It is certain that this tendency to splitting permitted entry of decay fungi into the inner untreated wood.

In the test section at 180 miles, approximately 75 per cent. of the 122 fluarized karri sleepers installed in 1929 were still serviceable after the 1944 inspection. The estimated average service life of 17·8 years for these sleepers may be conservative, and many individual sleepers are likely to exceed a total life of twenty years. Of those now remaining, the majority are either free from decay or only slightly affected on the heart face and it is likely that future renewals will be due to mechanical failure, splitting, brooming, and loosening of dog spikes. The reason for the excellent performance of these fluarized sleepers is not clear and, as the control set was approximately 11 miles distant, comparison with untreated sleepers cannot be made.

In the early years of the test a considerable growth of black fungous mycelium was noted on the untreated and fluarized karri sleepers in the Bowelling-Wagin section and was apparently similar to that reported

previously as common on powellized karri sleepers. According to reports, this surface growth was examined by the Plant Pathologist of the Western Australian Agricultural Department, who identified a *Heterosporium* sp. and a *Rhizoctonia* sp. However, the presence of this surface fungal growth did not appear to be correlated with early failure and disappeared in the later years of test.

The 97 untreated jarrah controls which were installed in 1934 in four groups continuous with each of the four karri test sections had required no renewals when inspected in 1944. Of these sleepers, 97 per cent. were still classed as "sound or slightly attacked" in 1944, and the remaining 3 per cent. as "attacked by decay or termites."

(iii) *Bolgart-Calingiri line.*

In this locality, three test sections each of 250 sleepers were originally installed, two sections being close together at 98 miles, and the third section 10 miles distant at 108 miles. In 1939 a deviation was constructed involving 149 test sleepers in the first section at 98 miles, and the 250 sleepers in the second test section at the same mileage. These sleepers were left in the old road bed out of traffic from March-November, 1939, when an inspection was made by the District Railway Engineer and an officer of the Western Australian Forests Department. Following this inspection, sleepers still considered serviceable were relaid at 104 miles and the test then continued.

In Table 2 the 101 sleepers which remained undisturbed at 98 miles have been treated as a separate test length.

The principal cause of failure in this locality was decay with some termite attack. The fungus *T. lilacino-gilva* was again common on decayed sleepers. Mechanical failure due to splitting, brooming, and loosening of dog spikes was not a common cause of renewal.

The first test section at 98 miles was particularly interesting, as the average service life of the 101 sleepers not disturbed by the deviation was only 10·7 years compared with 16·3 years for sleepers from the same length which were relaid in 1939 at 104 miles. This difference in service life, however, was obvious in 1939 before the sleepers were relaid, and was due apparently to a severe local hazard in the first portion of the test length. When inspected in 1939, 62 per cent. of the 101 sleepers in the first portion of the test section had been destroyed by decay and termite attack compared with only 18 per cent. of the remaining 149 sleepers. Apart from the occurrence of termite mounds near the track in the first portion of the test section there were no obvious reasons for this abrupt variation of result.

(iv) *Wyalcatchem-Bencubbin line.*

In this locality the 711 fluarized karri sleepers in test were distributed in two test sections approximately 13 miles apart, and gave an average service life of 13·6 years, ranging from 13·1-14·1 years in the two sections.

The principal causes of failure in the early years of the test were decay and termite attack, but slow mechanical breakdown due to severe brooming and deep end-splits occurred in the later years, and the majority of sleepers still serviceable in 1944 will fail ultimately from this cause rather than from decay. Of the 304 sleepers remaining in

Table 2.—Service Records of Fluarized Karri Test Sleepers in Western Australia.

Locality.	Mileage from Perth.	Date Installed.	Treatment.	No. of Sleepers Installed.	Renewals (all Causes) for Periods Ending June 30.						Final Inspection—June, 1944.			
					1929 to 1934.	1934 to 1936.	1936 to 1938.	1938 to 1940.	1940 to 1942.	1942 to 1944.	No. Condemned.	No. Still Serviceable.	Average Estimated Remaining Life (years).	Estimated Total Service Life.
Bowelling-Wagin	199	10/29	Fluarized karri ..	125	..	..	22	10	31	20	11	31	3·3	13·0
	191	,,	,, ,, ..	140	..	12	29	14	18	20	13	34	3·7	12·3
	186	,,	,, ,, ..	363	..	5	37	30	60	83	42	106	3·4	13·7
	180	11/29	,, ,, ..	122	..	..	3	3	5	12	8	91	5·0	17·8
	191	7/30	Untreated karri ..	107	15	24	48	20	..	..	..	..	..	6·3
	199	4/34	,, jarrah ..	24	..	..	..	..	..	..	..	24	+10	+20
	191	,,	,, ,, ..	25	..	..	..	..	..	..	..	25	+10	+20
	186	,,	,, ,, ..	24	..	..	..	..	..	..	..	24	+10	+20
	180	3/34	,, ,, ..	24	..	..	..	..	..	..	..	24	+10	+20
Boigart-Calingiri	98	11/29	Fluarized karri ..	101	..	20	23	18	13	..	9	18	3·6	10·7
	98, 104	,,	,, ,, ..	149*	..	5	10	14	..	..	21	99	4·4	16·3
	98, 104	,,	,, ,, ..	250*	..	24	27	95	..	..	29	75	3·6	12·4
	108	,,	,, ,, ..	250	..	28	12	22	18	79	2	89	4·3	13·7
Wyalcatchem	168	11/29	Fluarized karri ..	357	..	2	15	50	52	55	9	174	2·5	14·1
Benoubbin	181	,,	,, ,, ..	354	..	6	51	64	50	39	14	130	2·6	13·1

* Owing to construction of a deviation these sleepers were relaid at 104 miles in December, 1939.

TABLE 3.—INSPECTION RECORDS OF FLUARIZED KARRI TEST SLEEPERS IN WESTERN AUSTRALIA.

Locality.	Mileage from Perth.	Date Installed.	Treatment.	Number of Sleepers Installed.	Inspection Dates.	Percentage of Total Number Installed.					Destroyed (all causes).
						Sound or Slightly Attacked.	Affected by Decay.		Affected by Termites and Decay.		
							Attacked.	Severely Attacked.	Attacked.	Severely Attacked.	
Bowelling-Wagin	180–199	10/29 to 11/29	Fluarized karri	750	12/33	99	< 1	< 1	..	..	Nil
					6/35	88	5	3	..	..	4
					6/37	63	19	5	..	..	13
					6/44	10	23	2	..	..	65
	191	7/30	Untreated karri	107	12/33	62	7	17	..	1	13
					6/35	20	19	15	1	5	40
					6/37	4	8	7	..	1	80
					6/44	..	..	..	..	..	100
	180–199	4/34	Untreated jarrah	97	6/35	100	..	..	..	..	Nil
					6/37	100	..	..	..	..	,,
					6/44	97	2	..	1	..	,,
Bolgart-Calingiri	98–108	11/29	Fluarized karri	750	7/35	82	7	5	<1	<1	6
					6/37	35	33	12	<1	<1	19
					11/39	14	35	14	1	<1	35
					6/44	7	23	6	<1	<1	63
Wyalcatchem-Bencubbin	168–181	11/29	Fluarized karri	711	7/35	84	12	2	..	<1	1
					6/37	55	28	5	1	<1	11
					6/44	33	9	1	..	..	57

service in 1944, 230 were classed as only slightly affected by decay. However, many of these sleepers were estimated to have a remaining service life of only two to three years because of their mechanical condition, and a number of others practically free from decay were condemned because of failure to hold the dog spikes.

Termite attack was severe in the test section at 181 miles, and in the 1937 inspection was considered a more important cause of failure than decay. In Table 3, where the percentage of sleepers attacked by decay or termites is shown for each inspection, the full extent of termite attack in this locality is not clearly indicated. This is because many sleepers shown as "destroyed all causes" were severely attacked by termites. This attack was largely internal, the shell of treated wood remaining unattacked. Apparently, entrance of termites occurred through splits which exposed the inner untreated wood. At the 1944 inspection, termite attack appeared to have ceased almost completely in this test section, the reason not being evident.

6. Conclusions.

As a preservative treatment for karri sleepers, the fluarizing process has given a considerable measure of protection in Western Australia against decay and termite attack.

Although treated sleepers failed principally from decay, associated in some sections with termite attack, it is considered that the real weakness of the treatment lay in the shallow penetration of the preservative and the susceptibility of treated karri sleepers to develop end-splits, thus exposing the inner untreated wood to decay and termite attack. This mechanical fault of fluarized karri sleepers is clearly shown in the Wyalcatchem-Bencubbin sections where decay hazard is relatively low and where in the later years of test mechanical failure due to splitting, brooming, and failure to hold the dog spikes became an important cause of renewal.

As eucalypt timbers are generally refractory to pressure treatment, preservation processes in which penetration is obtained by diffusion offer a promising alternative and merit further investigation in Australia, where the problem of utilizing less durable eucalypt timbers for rail sleepers is becoming increasingly urgent. As many low durability eucalypt timbers are free splitting, it may be inferred that their successful preservative treatment for rail sleepers will be dependent not only on depth of penetration, toxicity and retention of preservative but also on treatment and/or methods of use designed to retard splitting and other mechanical failure.

7. Acknowledgments.

Acknowledgment is made of the co-operation of officers of the Western Australian Government Railways who were responsible for the installation of the tests, and who rendered every assistance during inspections.

Appreciation is also recorded for the assistance and co-operation of officers of the Western Australian Forests Department who inspected the Bolgart-Calingiri sections in 1939, and who assisted at all inspections in 1944.

It is also acknowledged that this report includes detail of inspections made by other officers of the Division and, in particular, Mr. J. E. Cummins, who was Officer-in-Charge of the Preservation Section until 1940, and was responsible for planning the tests.

The Sources of Agar in Australia.

*By E. J. Ferguson Wood, M.Sc., B.A.**

This paper is intended to summarize the present knowledge concerning the location and general habits of agar-producing seaweeds in Australia, and to act as a guide in the harvesting and preparation for manufacture of *Gracilaria confervoides.* It is probable that most of the statements regarding the preparation of *Gracilaria* will apply to other agar-producing seaweeds.

Agar is derived from certain seaweeds, all of which belong to the Rhodophyceae or red algae, in which it corresponds physiologically to the cellulose of higher plants: that is, it forms a constituent of the cell wall. Many of the red algae fail to yield an agar, but there is nothing in the literature to suggest why this is so. It has not so far seemed pertinent to this investigation to study the point. Of the species examined, those which yield satisfactory agars are, in the main, from the genera *Gracilaria, Gelidium, Pterocladia,* and *Eucheuma,* while the Russians add *Ahnfeltia.* Agaroids are yielded by *Chondrus, Grateloupia, Catenella, Gigartina, Phyllophora, Iridaea,* &c.

Sources of Agar in Other Countries.

In Japan the chief source of agar is the *Gelidium corneum* group, though other red algae, including *Gracilaria confervoides, Camphylaphora hypnaeoides,* and other *Gracilaria* species, are blended with the *Gelidium* to produce certain desired qualities. The quantity of agar produced in Japan depends on the severity of the winter to freeze the agar during manufacture, but latterly it has also been adversely affected by the pollution of estuaries by industrial waters. Artificial methods of propagation are stated to be used to augment natural supplies of the seaweed, but the actual position of the agar industry, and of the quantity of material available for manufacture in Japan, is not known. *Gelidium* is a rock-growing weed and is harvested in the estuaries by divers and by grapnels. The comparative ease of harvesting *Gracilaria* is an advantage to the Australian agar industry.

In Malaya, *Eucheuma speciosum* was used for agar.

Russia has, in the last few years, been producing agar from *Ahnfeltia plicata* at Vladivostock, from *Phyllophora* spp. at Odessa, and from *Phyllophora* and *Iridaea* at Archangel. The Russian material is chiefly an agaroid, although Kizevetter (3) claims that *Ahnfeltia* agar is of good quality.

America is using *Gelidium cartilagineum,* which is obtained by diving (1, 6), while New Zealand has begun using *Pterocladia lucida.*

In India some experimental work has been done with *Gracilaria* sp., and in South Africa with *Gracilaria confervoides, Hypnea specifera, Suhria vittata, Gelidium cartilagineum,* and *G. pristoides* (2). As far as the writer is aware, there is no commercial production in these two countries, although preparations are being made for the manufacture of bacteriological agar.

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Sources of Agar in Australia.

A paper is being prepared which will deal in greater detail with the properties of the agars and agaroids of Australian seaweeds.

In the meantime, it has been shown that *Gracilaria* and *Eucheuma* occur in sufficient quantity to warrant the establishment of an agar industry, and an increasing quantity of the *Pterocladia–Gelidium* group is being discovered. So far, however, *Pterocladia* and *Gelidium* occur mainly on rocky headlands, facing the open sea, where harvesting would be difficult or expensive. As this weed grows between high and low water, it could only be harvested at low tide in calm weather, and it is also difficult to harvest without removing some of the substrate with the holdfast.

Red algae are usually found in this country between high water mark (usually below half-tide mark) and about 4 fathoms, and certainly are not confined to regions below the brown algae as was formerly thought. Many of the reds grow on rocky substrate, some grow attached to shell or rocks, &c., in sand or mud, and some are epiphytic. From the point of view of cheap harvesting, those growing in sandy areas are most desirable, and it is for this reason that *Gracilaria confervoides* is regarded so highly. It can be harvested rapidly by mechanical means, especially as it occurs completely covering certain areas, quantitatively resembling a crop of lucerne. Also, as it is usually attached to solid material well below the sea bed, it is not likely to be destroyed by continued harvesting.

Prior to this research, studies on the red algae in Australia were limited to taxonomic work, often of limited accuracy. At the present time, taxonomic and distribution studies are in progress. It is necessary to summarize some findings on the more important agar-producing species without, however, making any final pronouncements on them.

Specimens of the *Gelidium–Pterocladia* group have been collected in a number of places, but only at Angowrie (near the mouth of the Clarence River) is the group known to be in commercial quantities. This may be largely due to a lack of quantitative study of our coasts. The economic possibilities of these species are still undetermined.

Hypnea musciformis is widely distributed and has been collected in most months of the year. It is very patchy, and has not been found in substantial commercial quantities. There is possibly sufficient material available for blending with other weeds or for providing Australia's needs of bacteriological agar. The cost of collection would be rather high.

Pterocladia lucida occurs in the Sydney area and on Rottnest Island amongst other places, but has not been found in sufficient quantity for commercial exploitation, although it is known that this weed gives an excellent agar in New Zealand.

A *Gracilaria* is found washed up on many beaches in Tasmania from King Island to Bicheno and D'Entrecasteaux Channel. In Storm Bay it grows on the scallop beds, but reports indicate that the occurrences do not warrant an agar industry in Tasmania. This seems to be a southern *Gracilaria* and may in fact represent more than one species.

Eucheuma (Pl. 10, Fig. 2) has been collected on beaches from Safety Bay to Dongarra in Western Australia, and possibly occurs farther north. It grows on rocky ledges of the reefs along the Western Australian coast. Recent surveys carried out under the auspices of this Division have shown a concentration of *Eucheuma* on the beaches at Dongarra, though a survey of the reef there by Miss A. Baird and Mr. G. T. Smith was disappointing. More recently heavy sheddings were found by our officers and others on Pelsart, Little Wallaby, and other islands of Houtmans Abrolhos. As a result of this work it is obvious that there is sufficient *Eucheuma* for at least a small agar industry in Western Australia, and such an industry now exists there.

Gracilaria confervoides (Pl. 9; Pl. 10, Fig. 1) is by far the most prolific species of agar-producing seaweed known to occur in Australian waters, and so far has been found mainly on the east coast. It was noticed by Mr. W. H. Pilmer at Bateman's Bay, New South Wales, at the beginning of the war, and recognized by him as a source of agar. The occurrence was brought to the notice of this Division by Mr. A. Laver, Fishery Inspector at Bateman's Bay, and work on the weed was begun. When inquiries were made by the Medical Supplies Committee regarding the possibility of supplies of agar being available in Australia, laboratory research was well advanced. In June, 1941, when a large occurrence in *Gracilaria* was discovered in Botany Bay by the writer and Mr. R. Bouchier of Cronulla, it was decided that a commercial industry was possible. In February, 1941, a small bed had been discovered in Port Hacking, and, later, a large bed was found in Middle Harbour. All these occurrences were in estuarine areas with a sandy bottom covering a mixture of sand and mud. When the survey was extended along the coast, the same environment was examined, and it was not until a bed was discovered in Lake Woolaweyah, near Yamba, New South Wales, by Mr. Phillips, of Yamba, that it was realized that the weed could grow in sand with a large admixture of mud. Closely following this came the discovery of an area of *G. confervoides* in Moreton Bay, Queensland, by Mrs. Valerie Jones, algologist to this Division. The weed is growing on a sandy bottom with some admixture of mud.

A very recent survey of Moreton Bay made by the writer with Mr. B. Rose, an experienced seaweed harvester, has shown that large areas there yield *Gracilaria confervoides*. The Moreton Bay beds occur mainly between the Old Pile Light and the entrance to Canaipa Passage, and will require rather larger vessels and stouter gear than the New South Wales beds. However, satisfactory drying grounds are reasonably close to the beds, and this will be a decided advantage. The quality of the weed if properly collected and treated is excellent.

A search of similar areas to Lake Woolaweyah resulted in a number of records from Eden to the Tweed River, but the writer now thinks that there are at least three varieties of *Gracilaria* which must be distinguished from the economic point of view. The first is a short weed growing on whelks, cockles, and at times on sticks, stones, and other substrates. This weed is little-branched, and under observed conditions does not grow longer than 6 inches. It is known to occur in Wapengo Inlet, Tuross Lake, Cuttagee Lake, Moruya River, Durras Lake, Lake Conjola, Crookhaven River, Camden Haven River, Queens' Lake and Watson Taylor Lake, Bellinger River (South Lagoon), Brunswick

River, and Terra Nora Lake. Local opinion in these areas holds that this is a fixed variety, and the writer's observations support this. There is, however, a possibility that, under certain conditions of salinity, &c., this weed might develop normally, but this possibility must be disregarded in assessing the amount of weed available for manufacture.

The second variety is that growing in Cathie Creek (Lake Innes) and in the Clarence River Broadwater in New South Wales, and in the Swan River, Western Australia. This variety looks like a half-grown sample, and grows up to 12 inches long. At Cathie Creek and the Broadwater, fishermen report that the weed does not grow past the stages already observed by the writer, and this is confirmed by seasonal observations. These areas are normally brackish rather than salt, and this type appears to have a greater tolerance for fresh water than the third type. On account of its relatively low yield of agar by the usual methods and of the difficulty of extraction, this variety must also for the time be disregarded from a commercial standpoint. Tseng (private communication) also records two American varieties known as *G. confervoides* which appear to resemble in their properties the last two varieties mentioned here.

The third variety is found usually in estuaries, and grows from 18 inches to 12 feet long. On sandy bottom it is usually red, straw-coloured, or green, and on dark bottom (some mud) is brown to purple. Evidence at Lake Woolaweyah and Shaw's Bay, on the Richmond River, suggests that this type is sensitive to reduced salinity. In 1940, a fisherman at Bateman's Bay told the writer that the weed disappeared in wet years, and was at its best in drought periods. There has been no harvest of *Gracilaria* in this district until this year (1944) since the coastal drought broke in 1943. At Shaw's Bay, a crop was ready for harvesting when a heavy flood occurred. A week later, there was left only "about 3 inches of substance like over-soaked glue," and another week later when the writer arrived this had disappeared. This bay is open to the sea only by percolation through a breakwater, and the weed itself could not get through. These observations were made by Mr. W. Packenham, of the State Fisheries Department. During this flood, the writer saw some half-grown *Gracilaria* on the beds at Lake Woolaweyah, but a fortnight later this weed had left the beds and rolled into gutters on the flats. This is the type of weed used in the commercial manufacture of agar in New South Wales. In 1943 between 200 and 250 tons of the dried weed were harvested for agar production, mainly from Middle Harbour, Port Jackson, and Botany Bay. Detailed monthly surveys have been made of these two areas and of Port Hacking in order to study the seasonal growth and the effects of harvesting, and to time the harvesting so as not to injure the beds. This work is continuing, and the results will be published later. In 1944 the Botany Bay crop was rather heavier than in 1943, and that in most of Middle Harbour was less owing to a storm just as the weed was maturing.

It has been found in the course of surveys that *Gracilaria* of types 2 and 3 is usually torn off the beds in heavy storms, at whatever stage of growth it may be. This weed may wash ashore and, in such cases, is usually too heavily mixed with other weeds (*Zostera, Posidonia,* &c.) and with sand, to be of any commercial value. At other times it is rolled into pockets or gutters, and may exist in such places for a considerable period. There is nearly always a good deal of *Gracilaria*

off Edwards Beach in Middle Harbour, and, for a time, this was regarded as a bed. It is now considered that the weed from other parts of the bay is swept into this area and remains there. It is usually broken into short pieces, and is occasionally washed ashore. Tests show that it does not lose its agar quality by lying loose on the bottom for some time. Certain areas at Yamba and at Botany Bay yield a quantity of weed which can be traced to other areas, and is concentrated by the weather and the configuration of the bottom. In unfavourable weather, with the tides setting strongly out, much loosened weed goes to sea and is lost. Depth measurements taken monthly have shown considerable siltation and denudation on some of the beds, and this, coupled with the effect of storms, suggests that fairly intensive harvesting should not reduce the supply of weed. When conditions are favourable, the weed grows with amazing rapidity, to the extent of inches per week, and even in closely harvested areas of Middle Harbour, there seems no evidence of a diminution of growth, and in some areas which are regularly harvested, e.g., Balmoral, there seems to be an increase of growth.

In Middle Harbour and Botany Bay, *Gracilaria* can usually be found attached to the tubes of Turbellarian worms. In Botany Bay it is also attached to pebbles, and in Lake Woolaweyah to whelks and cockles. It is possible that, unless care is taken, the removal of these animals with the weed may cause a diminution in yield, but this would depend on the rate of reproduction of the animals and the rate of attachment of *Gracilaria* spores. *Gracilaria* has frequently been found apparently growing where it has been trampled in by gatherers, and it continued to grow for a time in aquarium jars. It is necessary for the weed to be anchored by some means to ensure continued growth. Twisting filaments round small stones or pieces of fibro-cement and planting these in the sand has proved successful in cultivation experiments. Culture experiments also show that the plant spores adhere readily to a suitable cultch, e.g., worm casts, shells, rock, and grow out to start a crop. The catch seems to occur in late summer and autumn. Latest results of the writer's experiments show that cultivation by these two means can be adapted to commercial practice if required. Evidence is that unless the weed has an anchor of some sort, it is swirled out of the sand by tides and surges, and soon disappears. On the other hand, whelk beds do not necessarily mean the presence of *Gracilaria*. There seem to be other factors necessary for growth. There is not yet enough evidence to clarify the position, though salinity is certainly one of the factors. The weed is definitely seasonal, growing rapidly in the spring to yield a harvest in September to October and on through the summer to January. There may also be an autumn crop.

From observations at Edwards Beach, Lake Woolaweyah, and in Botany Bay, it seems that it should be possible to keep *Gracilaria* in pens in the water when the crop is heavy, or when wet weather is likely to ruin a drying crop. Only a few marine bacteria digest agar, but, in fresh water, bacterial action is rapid, and decomposition soon occurs.

It is considered that the known sources of weed near Sydney will continue to yield approximately 300 tons of dried *Gracilaria* annually. This estimate is based on two years' harvesting, and does not allow for the weed which is washed away during bad weather or through delays in harvesting.

A large area of good *Gracilaria* exists near Yamba on the Clarence River, and other areas are known in Moreton Bay. At Yamba, three men with some assistance harvested 12½ tons of weed in six weeks, but this was rather late in the season, and does not give a clue to the productivity of the area. All that can be said is that the possible yield is greatly in excess of this. No indication can be given at present of the amount of weed harvestable from Moreton Bay. If the known areas yield tonnages comparable with those near Sydney, the Moreton Bay area should provide more than the New South Wales areas.

As a last resort, it is possible to treat the *Gracilaria* from the Clarence River Broadwater and from the Swan River, in Western Australia, so as to get a very good agar. Meanwhile it is believed that the Western Australian demand can be adequately supplied from the *Eucheuma* areas of the Dongarra-Abrolhos region.

The Environment of *Gracilaria confervoides*.

Studies on the possibilities of culturing *Gracilaria* are in progress, but at the present time little can be said on the subject. The writer has found that, although *Gracilaria* appears to grow in sand or mud and sand, it almost invariably is attached to some object which is usually several inches, or may be a foot, below the surface. At Yamba the weed is attached to whelks, mainly to small ones; at Middle Harbour, to the casts of a Turbellarian worm; at Doll's Point, Botany, to sandstone pebbles; at Lawrence, to trachyte; at St. George's Basin and Tuggerah to wood, or even to Casuarina cones; in the channel behind Towra it adheres to oyster shell, and has been found on cockles in Port Hacking. On one occasion it was found attached to an iron stake, to slag, and to experimentally placed fibro-cement. The adherences to these objects is feeble, sometimes by rhizoids, and sometimes by a single attachment. From the point of attachment the weed grows upward through the sand or mud to the surface where branching occurs. If the weed is covered by sand at this stage, it will grow through again and branch at the new surface. It appears to grow very rapidly in season, and a bed may show relatively little one month, and be ready to harvest the next. The writer considers that these sudden appearances may be due to rapid growth, to uncovering of buried weed, or to a combination of both. At Doll's Point, a weed a foot to eighteen inches long was uncovered during heavy weather in May-June, 1944. It was not visible on June 1, but was collected on June 9. The stone to which it was attached was still 6 inches below the sand. For this reason, there seems little possibility that harvesting will adversely affect the beds unless machines are used which dig more than 3 inches into the bottom. If, however, a large number of worm casts, whelks, &c., were found in the weed it would suggest that there was need for caution in harvesting, and possibly for some measure of control, as has been mentioned previously.

It is of course inadvisable to harvest immature weed, as the yield would be greatly lessened, and, possibly, the yield of agar in the weed might be reduced.

Weed that is growing in turbid areas where there is a considerable amount of suspended mud is difficult to clean. The usual method of washing by hand is not very effective, and the material at times arrives

at the plant with a considerable amount of colloidal clay adhering to it. Washing with beaters will help to remove it, but it is possible to use this weed by buffering the agar liquor with acid sodium phosphate to overcome the alkaline buffer action of the clay.

Methods of Harvesting.

The method used for harvesting *Gracilaria* is to drag two or more rock anchors behind a dinghy, a man hauling each of these alternately. Usually two dinghies are towed by one motor-boat, and three men form a seaweed crew. The rock anchor consists of a piece of 2-in. pipe, through which pass four pieces of iron rod, forming a ring at one end and bent back to about 70 degrees to the pipe at the other. A rope is attached to the ring and the anchor is towed so that two prongs scrape along the bottom, the other two being 4 to 6 inches above. The weed catches on the prongs and is easily hauled aboard. As much as a hundredweight of wet weed may be pulled up on one rock anchor, and as much as 8 tons (equal to 1 ton of dry weed) may be gathered in this way by three men in a week. A series of rake teeth on an endless chain has been designed by the writer, but has not yet been used. There is room for greater mechanization in the harvesting, as the bottom is usually fairly smooth and sandy, and the weed fairly uniformly spread over the beds.

Eucheuma speciosum and other *Eucheuma* species are harvested at present by collection from the foreshores. When the location of the actual beds is known it is probable that recourse may be had to diving; or some type of rock grapnel may be used. It is obvious that this weed will not be so easy to harvest as *Gracilaria*. The problem of the holdfasts which usually retain large portions of the rocky substrate will be serious from the point of view of manufacture and of replenishing the beds. It will probably be necessary to cut the weed above the holdfast. For these reasons *Gracilaria* is a cheaper raw material.

Preparation of Raw Material for Manufacture.

The wet seaweed, as collected, is not suitable for agar manufacture. Experiments with *Gracilaria* have shown that an extract of the undried seaweed does not gel. For this reason, as well as on account of transport, drying is necessary. Bleaching is also considered essential by some manufacturers, although the writer does not agree with this.

In practice, harvested seaweed is dried on the grass or on netting racks (Plate 11), and at times *Gracilaria* is dried on poles. It has been found that weed dried on grass bleaches naturally and more rapidly than that placed on racks. The reason for this is not clear. In an experiment to study the methods of drying and bleaching the following tests were made:—

> A quantity of uniform *Gracilaria confervoides* was divided into a number of 16-lb. lots. These were treated as follows:—
>
> 1. Placed on rods in an ordinary fish-smoking kiln. A coke brazier was lit and kept burning for 24 hours, giving a dry bulb temperature of 30-40°C. and a relative humidity of 30-40 per cent. The weed had dried in this period and weighed 2 lb. 3 oz., i.e.,

13·7 per cent. of the original weight. It recovered moisture in the atmosphere to weigh 15 per cent. of the original weight with a moisture content of 17·8 per cent.

2. Placed on rods in a tunnel drier, and dried with partial recirculation at 55°C., with the temperature rising to 60°C. Relative humidity was 40 per cent. and fell slowly after three hours. The weed was too tightly packed and sweating occurred. The weed was wet in places after 24 hours. After turning, it dried in 36 hours. The dry weed weighed 2 lb. 4 oz., or 14·06 per cent. of the wet weight. It absorbed moisture until it weighed 16·4 per cent. of the original weight with a moisture content of 16·8 per cent.

3. Dried in a tunnel drier at 45°C. in 24 hours. Yield 2 lb. 6 oz., or 14·8 per cent. of the wet weed.

4. Placed on a wire rack and allowed to dry and bleach under natural conditions. This weed dried in two days, but required 21 days to bleach. At the end of this time bleaching was not very satisfactory. The yield was 1 lb. 4 oz., or 7·8 per cent. of the wet weight.

5. Placed on a wire rack and watered each night with a spray pump. This weed also dried in two days, and bleached in 21 days. The yield was 1 lb. 5 oz., or 8·2 per cent.

6. Placed on a wire rack and dried in the sun, but covered from rain at night. It dried in two days and gave 2 lb. 9 oz., or 16 per cent. of the wet weed, with a moisture content of 17 per cent.

It should be noted here that bone-dry weed will rapidly take up moisture, even in fine weather, to approximately 17 per cent., unless the weed has been bleached. Thus, a moisture content of 20 per cent. would be allowable on unbleached or bleached weed, and the commercial price should be based on this. Samples 7, 8, and 9 were prepared as were 1, 2, and 3, but were subsequently bleached respectively—thinly strewed on grass and turned every second day; placed over wire netting; placed on a wire fence.

The weed on grass bleached in seven days and yielded 1 lb. 3 oz., or 7·4 per cent.; that on netting took 21 days and yielded 1 lb. 3 oz., or 7·4 per cent., and that on the wire took six weeks and yielded 1 lb. 4 oz., or 7·8 per cent.

The loss of weed in bleaching was of the order of 50 per cent.

Samples of each of the preparations described above were tested for agar content and gel strength by a standardized method. The results were—

(1) gave a 25 per cent yield of excellent agar with a gel strength by penetrometer A of 67·3g.

(2) gave a 24·4 per cent. yield with a gel strength of 68·2g.

(3) gave a 31·2 per cent. yield with a gel strength of 63·6g.

(4) gave a 27 per cent. yield with a gel strength of 52·1g.

(5) gave a 27·5 per cent. yield with a gel strength of 55·2g.

(6) gave a 38 per cent. yield with a gel strength of 65·5g

(7) gave a 25 per cent. yield with a gel strength of 56g.

(8) gave a 26·5 per cent. yield with a gel strength of 39g.

It should be noted that the highest gel strengths were from 1, 2, and 6, none of which were bleached. The bleached agars gave a significantly poorer gel, although the grass-bleached agar gave the best result for bleached weed. The yields of 3 and 6, both unbleached, were the two highest. This means that bleaching results in a halving of the yield in terms of wet weed, and also if the weed is not sweated as in 1 and 2, reduces both yield and gel strength in the remaining weed. The reason adduced for this is the loss in bleaching of the water-soluble fraction described by Lipatov and Morozov (4, 5).

It has been shown in other experiments that unbleached weed can be treated so as to yield a colorless gel by means of activated carbon, and that even bleached weed will not do so without carbon. The conclusion is therefore that bleaching is undesirable. It has been averred by some manufacturers that unbleached weed is difficult to store, but the writer has used unbleached weed that had been in storage for eighteen months and had not deteriorated appreciably in that time. The storage was by no means ideal, as water was running over the floor in close proximity to the weed. A manufacturer has used unbleached weed after eighteen months in store under poor conditions; the agar was excellent. It will be seen from the figures given that unbleached weed will take up about 20 per cent. moisture from the atmosphere, and the writer considers, as mentioned above, that payment should be on a 20 per cent. moisture basis. If moisture samples are taken when the weed is weighed, the value of the weed can be kept constant. Care should be taken in sampling that both the outside and the centre of the bale are included, as there may be a steep moisture gradient. Bleached weed may have a moisture content of 17-20 per cent. absorbed from the atmosphere.

Artificial drying in a tunnel drier was not highly successful. A series of tests have shown that the yield of artificially dried weed is the same as that of sun dried weed, but the gel strength of artificially dried weed was greater by an amount that exceeded the experimental error (59·4g. against 44·9g.). Unless the weed was very thinly spread, sweating occurred, and in any case the time of drying was not lessened sufficiently to warrant the expense.

When the weather is bad for a week or more many tons of weed may be lost, as it deteriorates if soaked by fresh water for such a period. It has been found that weed thinly spread on racks does not spoil so much even after a fortnight's rain. If the racks were portable they could be stacked in a shed warmed by a slow fire in order to minimize losses in the wet season. Such a shed might easily be built in each harvesting area in case of emergency.

These experiments show the fact previously noticed in the field, that *Gracilaria* bleaches much more rapidly and evenly on the grass than on racks. It was thought that this was caused by dew moistening the weed each night, but the weed on racks in lot 5 watered each night did not bleach more rapidly than the natural weed on the racks

The Mechanism of Bleaching.

Laboratory experiments on bleaching carried out by the writer have given the following results:—Samples of weed fresh from the sea were placed in sterile test tubes, each fitted with a cotton-wool plug, and

having at the bottom either a layer of moist sterile sand or a layer of calcium chloride covered by a cotton-wool wad. Table 1 gives the results—

TABLE 1.—CONDITIONS REQUIRED FOR BLEACHING.

—	Co_2.		Air.	
	Moist.	Dry*.	Moist.	Dry*.
Tubes in dark	No bleach	No bleach	No bleach	No bleach
Tubes in sunlight	4 hours	8 hours	24 hours	24 hours

* Drying incomplete—weed tended to retain moisture despite the calcium chloride.

From a number of sunbleached samples of *Gracilaria,* fungi were isolated but no bacteria. The fungi were: a frequent *Alternaria* sp., a less frequent *Mucor,* and an *Aspergillus.* Spores were suspended in water and sprayed on to fresh *Gracilaria* in test tubes. Duplicate tubes were exposed (*a*) to sunlight , and (*b*) in a 26°C. incubator in the dark. All the tubes exposed to light bleached in 48 hours, those in the incubator did not, but bleached in 24 hours on exposure to light. As the controls behaved similarly it was concluded that the fungi were secondary invaders. They were completely outgrown by bacteria during an attempt to re-isolate them.

Water retting experiments were tried at pH ranging from 4 to 9·5, but it was found that bleaching only occurred near the surface exposed to sunlight. Various bacteria, including many *Proteus vulgaris* strains, were isolated, but it could not be shown that these were the cause of bleaching. All the tests became acid (pH 4), and butyric acid fermentation finally set in with evolution of CO_2 which may have assisted bleaching. From this and the previous experiments it was concluded that sunlight, moisture, and a certain concentration of carbon dioxide are necessary for "sun-bleaching." It has also been found that completely bleached weed has a pH of 6 to 6·5 and in the presence of moisture becomes still more acid, so that a 12-hour washing brings the pH below 5 and butyric acid fermentation is noticeable. The agar yield drops rapidly, as pH 5 is the lowest pH at which agar can be safely boiled without hydrolysis.

References.

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6. Tzeng, C. K.—Utilization of seaweeds. *Sci. Monthly* **59**: 37 (1944).

NOTES.

A Device for Expediting the Measurement of Fibre Diameter.

(*Contributed by E. H. Mercer, B.Sc.,* and W. I. B. Smith, B.Sc.**)

The usual method of determining the average diameter of the fibres from a sample of wool involves the measurement of the images of sections of the fibres projected microscopically at 500 magnification. This is a time-consuming process and the present device was developed to shorten it.

In essence it is an elaboration of the simple cartometer, which is used to measure lengths on maps, &c., by counting the number of revolutions of a wheel rolled over the surface.

The rolling wheel in this case is carried at the end of a convenient pen-like handle (see Fig. 1) and is rolled in turn over the projected images of the fibres. A line ruled across a piece of "perspex" extending in front of the wheel facilitates accurate setting on the edge of the fibre image.

The total length of such paths as A-B (Fig. 1) divided by the number of fibres gives the average diameter. The lengths A-B are continuously added by a contact on the rolling wheel which actuates an ordinary telephone register recording the number of revolutions. The number of fibres measured is recorded at the same time by a contact in the handle which closes another circuit when the wheel is pressed on the table.

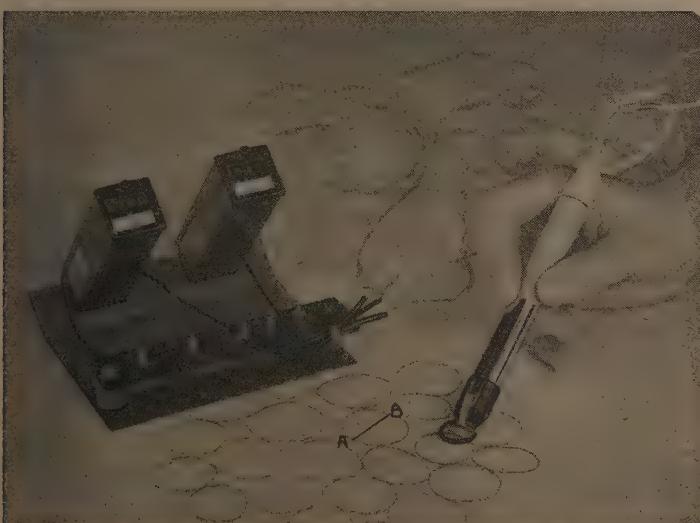


Fig. 1.

The device only determines average diameter, but for many purposes this is useful. It may also be used to determine average lengths arising in other investigations.

* An officer of the Council stationed at the National Standards Laboratory, Sydney.

Flax Research Laboratory.

The Flax Section of the Division of Forest Products has recently been merged into the Flax Research Laboratory, which the Council is establishing in co-operation with the Flax Production Committee of the Department of Supply and Shipping. The laboratory is to investigate problems associated with the processing of flax fibre and the ultimate spinning of the fibre into flax yarn. It is proposed that the equipment of the laboratory shall include experimental retting tanks, an air-conditioned room for the investigation of processing problems on scutching, and other machinery and facilities for chemical, physical, and biological studies associated with the processing of flax fibre. It is hoped that an experimental flax spinning plant will also be provided.

The laboratory is to be staffed and operated by C.S.I.R., and its research activities will be guided by an advisory committee comprising representatives of the various interests concerned.

Mr. W. L. Greenhill has been appointed Officer-in-Charge of the Laboratory. Mr. Greenhill was formerly in charge of both the Flax and the Timber Physics Sections of the Division of Forest Products.

Interdivisional Conference on Toxicology.

Officers from about ten of the Council's Divisions and Sections met in Canberra from July 2-6 to discuss many problems which come under the general heading of toxicology, and which are of importance in Australia's primary and secondary industry. Among the subjects discussed were those relating to the control of plant and animal insect pests, fungal and bacterial diseases of plants, noxious weeds, wood-destroying fungi, and the prevention of food spoilage.

In all these fields of work, which are centred in different Divisions, there are many difficulties which have certain aspects in common and which can only be resolved by discussions such as the present one. Particular attention was given to progress in the Council's work on D.D.T.

This is the second such conference which has been arranged, the first being held in Melbourne in July, 1943. It is proposed to hold a third conference in Sydney within the next eighteen months.

Visit of Wool Textile Advisers.

The Council has been fortunate in obtaining the services of several leading overseas textile authorities to assist in the formulation of plans for the comprehensive programme of wool textile research, envisaged in the *Wool Use Promotion Act* 1945.

Dr. A. C. Goodings, Director, Textile Section, Ontario Research Foundation, Toronto, and Mr. B. H. Wilsdon, Director of the Wool Industries Research Association, Torridon, Leeds, have just completed

a series of visits embracing all States of the Commonwealth, in an endeavour to make themselves familiar with the wool processing and manufacturing industry in Australia, and such research work on wool as is now being conducted. Mr. W. R. Lang, Officer-in-Charge, Scientific Research Department, Gordon Institute of Technology, accompanied them on their tour.

Professor J. B. Speakman, Textile Industries Section of the Faculty of Technology, University of Leeds, has recently arrived in Australia.

Recent Publications of the Council.

Since the last issue of this *Journal,* the following publication of the Council has been issued:—

Bulletin No. 184.—"Fellmongering Investigations," by F. G. Lennox, D.Sc., Margaret E. Maxwell, M.Sc., and W. J. Ellis, A.S.T.C.

This Bulletin is made up of twelve papers. The first of these reviews methods at present employed in the fellmongering industry, and the remainder describe the biological and chemical research work on fellmongering carried out in the Biochemistry Section of the Division of Industrial Chemistry during the past four years.

The work was undertaken by the C.S.I.R. because some of the methods now employed in the fellmongery damage the skin and the wool and produce noxious odours; moreover, the changes underlying the processes employed have never been adequately studied, and the demand on local fellmongers has greatly increased in recent years, mainly owing to the loss of French markets for skins.

The two processes employed in the industry in Australia are sweating and painting. The former depends on the loosening of wool by bacterial action, and the latter depends on the wool-solvent action of a chemical depilatory. So far, the research has been restricted mainly to the former.

The investigations have only been made possible by the development of a method of measuring the pull necessary to loosen the wool, which is described in the second paper of the series. Later papers deal with the bacteria responsible for wool loosening during sweating, and others deal with the influence of the soaking operation and the temperature of the sweating chamber on the rate of sweating; the importance of controlling the temperature of the sweating chamber during sweating and of keeping the humidity as near saturation as possible, is stressed. In view of the liberation of ammonia from sheepskins during sweating—the ammoniacal odour being very familiar to those engaged in the industry—investigations were undertaken to determine its effect on skins, and it has been demonstrated that exposure of skin to ammonia gas produces almost complete loosening of the wool within a few hours. The effect of this and of other fellmongering processes on the wool roots and on the skin tissues has been studied by the examination of skin sections under the microscope.

A new method of recovering wool from heads, shanks, and skin pieces, by heat-skrinking the material in water and then dissolving the skin tissues in a protein-digesting ferment or enzyme, is described in paper 11. Some of the major problems of the industry to-day are due to wool damage, and high labour costs involved, and to emission of a putrefactive odour during the recovery of wool from scrap material by existing methods of "picing." Special interest therefore attaches to this new process which should be very helpful to the industry if large scale tests support laboratory findings.

Forthcoming Publications of the Council.

At the present time, the following future publications of the Council are in the press:—

Bulletin No. 185.—"Studies on the Mitchell Grass Pasture in South-Western Queensland. 2.—The Effect of Grazing on the Mitchell Grass Pasture," by R. Roe, B.Sc. (Agric.), and G. H. Allen, Dip. Agric. (Lawes).

Bulletin No. 186.—"The General Ecological Characteristics of the Outbreak Areas and Outbreak Years of the Australian Plague Locust (*Chortoicetes terminifera* Walk.)," by K. H. L. Key, M.Sc., Ph.D.

Bulletin No. 187.—"Alcohol: Its Place in Synthetic Organic Chemical Industry," by H. H. Hatt, B.Sc., Ph.D.

Bulletin No. 188.—"A Soil, Land-Use, and Erosion Survey of County Victoria, South Australia, including the Hundreds of Belalie, Whyte, Reynolds, and Anne and part of the Hundreds of Caltowie, Yangya, and Bundaleer," by C. G. Stephens, M.Sc., R. I. Herriot, B.Ag.Sc., R. G. Downes, M.Agr.Sc., T. Langford-Smith, M.Sc., and A. M. Acock, B.A., D.Phil.

Bulletin No. 189.—"Soils of the Berriquin Irrigation District of N.S.W.," by Robert Smith, B.Sc. (Agric.).

Bulletin No. 190.—"Foundry Sand Resources of South Australia," by H. S. Cornelius and H. A. Stephens, B.Sc.

Bulletin No. 191.—"Studies of the Physiology and Toxicology of Blowflies. 10. A Histochemical Examination of the Distribution of Copper in *Lucilia cuprina*. 11. A Quantitative Investigation of the Copper Content of *Lucilia cuprina*," by D. F. Waterhouse, M.Sc.

Bulletin No. .—"Investigations of Guayule (*Parthenium argentatum* Gray) in South Australia," by R. L. Crocker, M.Sc., and H. C. Trumble, D.Sc., M.Agr.Sc.

Bulletin No. .—"Post-Miocene Climatic and Geologic History and its Significance in Relation to the Genesis of the Major Soil Types of South Australia," by R. L. Crocker, M.Sc.

PLATE 1.

Reactions of Tasmanian Bismark and Brownell Potatoes to the Commoner Virus Diseases (See page 209).

FIG. 1 (above).—Faint mottle typical of virus X on Bismark under conditions favourable for the manifestation of symptoms.

FIG. 2 (below).—Virus X from Brownell on President potato. Symptoms of the same type and intensity were evident on the Brownell plant from which inoculations were made.

PLATE 2.

FIG. 1.—Mottle and slight crinkling produced by virus A on Bismark under conditions most favourable for the production of symptoms. Partial or complete masking may occur under field conditions.

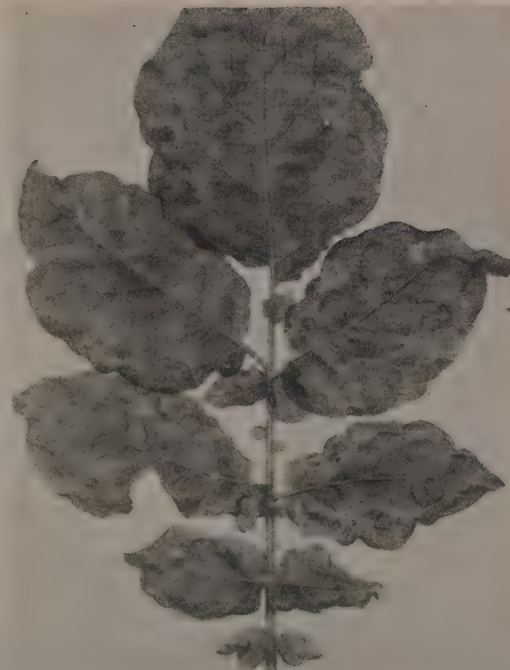


FIG. 2.—Leaf of a potato plant (variety Carman) showing crinkle and mottle produced by a composite infection with viruses X and A. The leaflets on a leaf of Bismark are more pointed, otherwise this photograph might pass for a picture of a Bismark leaf with advanced symptoms of crinkle.

FIG. 3.—Top leaves of a potato plant showing the rugosity and downward cupping and curling of the leaflet tips, characteristic of infection with virus Y. Contrast with the crinkled leaf in Fig. 2, the leaflets of which remain relatively flat, while their outline is distorted. The variety is Western Australian Delaware (Earliest-of-All), but similar effects are produced on Bismark and Brownell.

PLATE 3.

Reactions of Tasmanian Bismark and Brownell Potatoes to the Commoner Virus Diseases (See page 209).

FIG. 1.—Two Bismark potato plants, left healthy, right infected with leaf roll. The contrast in size and vigour may be even greater than is shown here. The leaves on the diseased plant are not severely rolled, but the foliage is harsh, stiff, and lighter in colour than normal. Infection rarely occurs in the field.

FIG. 2.—Two Brownell potato plants, left healthy, right infected with leaf roll. The dwarfing of infected plants is often *less* severe than in this instance. The leaves are harsh, the lower ones rolled, the upper ones stiff, rugose, yellow green in colour, and carried at an acute angle to the stem. Leaf roll is the principle cause of degeneration in Brownell.

PLATE 4.

The Effect of Flax Straw Maturity on the Microscopic Structure and Dimensions of the Ultimate Fibres (See page 225).

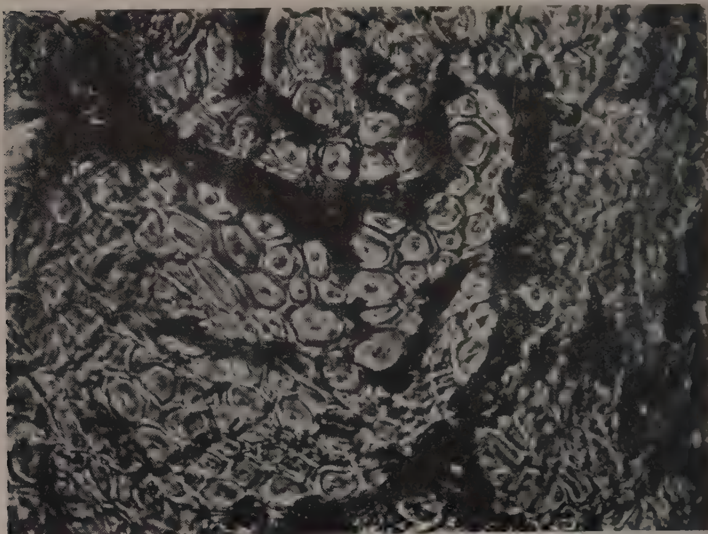


Fig. 1.—Ultimate fibres from 3rd Harvest. (× 430.)

Fig. 2.—Ultimate fibres from 7th Harvest. (× 430.)

PLATE 5.

The Effect of Flax Straw Maturity (See page 225).

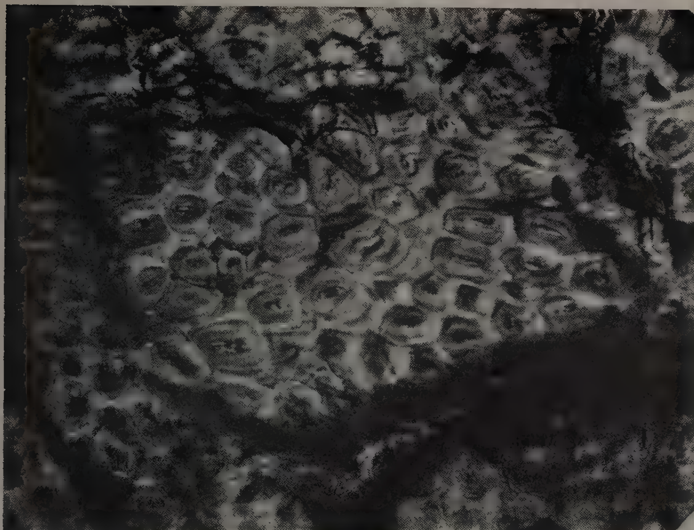


Fig. 1.—Ultimate fibres from 12th Harvest. (× 430.)

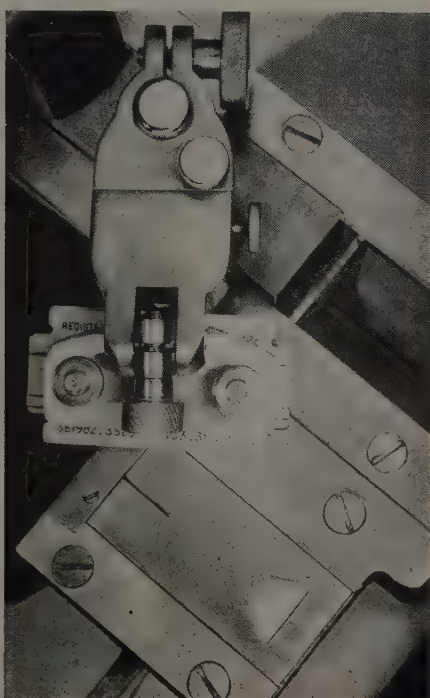
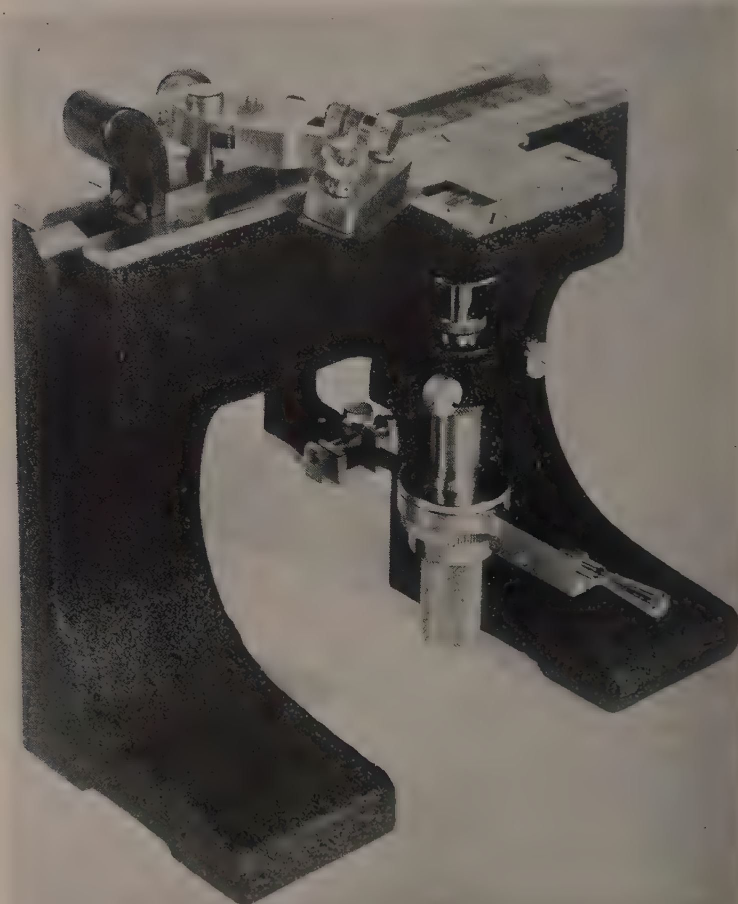


Fig. 2.—Fibre microtome, showing razor blade holder, also fibre in position in the slot.

PLATE 6.

The Effect of Flax Straw Maturity on the Microscopic Structure and Dimensions of the Ultimate Fibres (See page 225).



Fibre microtome showing complete instrument.

PLATE 7.

A Note on Vegetative Propagation and Tree Form in *Duboisia* Spp.
(See page 230).

Cuttings of *Duboisia myoporoides* six weeks after setting. (1) control; (2) immersed for eighteen hours in 1 per cent. aqueous solution of "Hortomone A" prior to setting; and (3) similarly immersed in 2 per cent. solution of "Hortomone A".

PLATE 8.

A Note on Vegetative Propagation and Tree Form in *Duboisia* Spp.
(See page 230).

FIG. 1.—Contrasting young tree types of *Duboisia Leichhardtii*.

FIG. 2.—Vegetative propagants of contrasting young tree forms of *Duboisia Leichhardtii*.

A_1, A_2, from tree A in Fig. 1; B_1, B_2, from tree B in Fig. 1.

PLATE 9.

The Sources of Agar in Australia (See page 263).

FIG. 1.—*Gracilaria conferroides* mixed with *Posidonia* on Kurnell Beach, N.S.W.

FIG. 2.—*Gracilaria conferroides* showing habit of growth.

PLATE 10.

The Sources of Agar in Australia (See page 263).

FIG. 1.—*Gracilaria confervoides* attached to rock. This was part of a field culture experiment. Note the fruiting bodies on the extreme right.

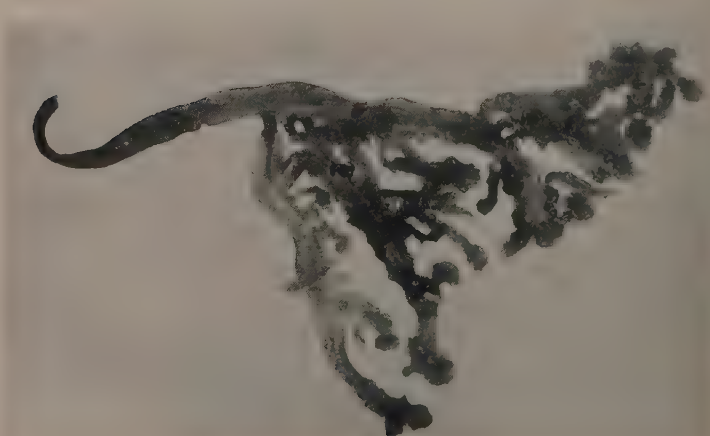
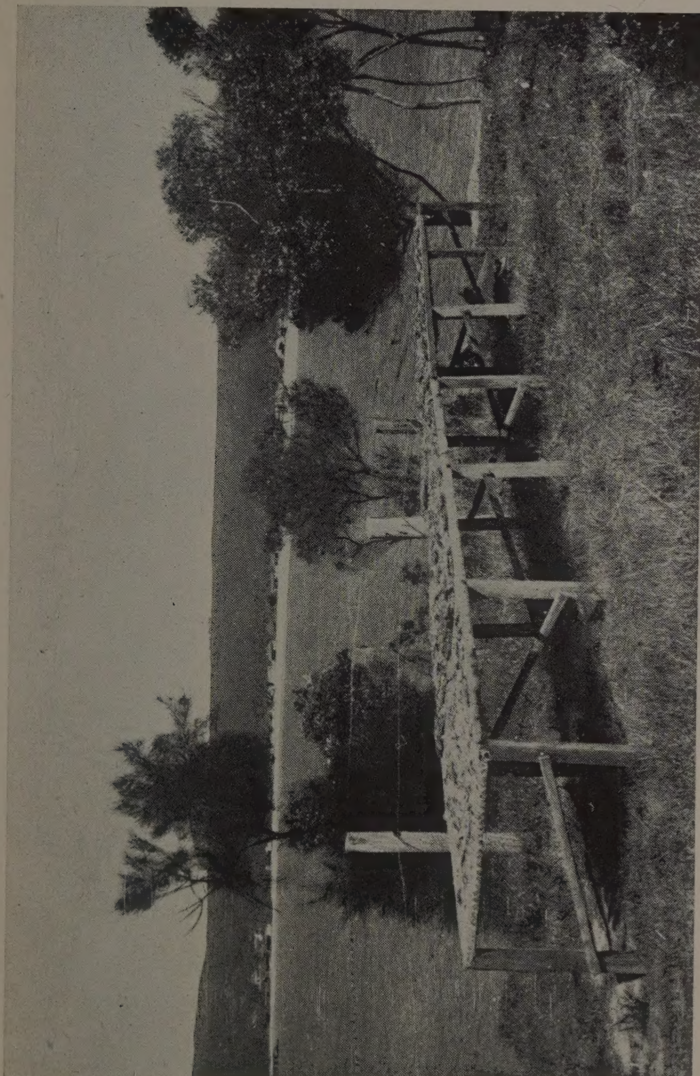


FIG 2.—A beach sample of *Eucheuma*

PLATE 11.

The Sources of Agar in Australia (See page 263).



Gracilaria drying on racks at Cronulla.

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